

Who says what drugs to license?

Last week's decision to end the manufacture of a drug widely used in pregnancy raises awkward problems. Part of any solution should be that regulatory authorities are joined in legal suits.

THE old motto "give a dog a bad name and hang him" seems to have worked with the drug called Debendox (in the United Kingdom) or Bendectin (in the United States). Last week, the Merrell-Dow organization announced that it had decided to abandon production of the material, used widely for the palliation of morning sickness in human pregnancy. The company insists that its product is safe, and that the incidence of malformations among children born to women taking the drug is entirely within the normal range, an opinion that has been confirmed by the formal studies of the side-effects of the drug since it was first put on the market 27 years ago. This does not, of course, imply that no malformed children will turn up among the offspring of women treated with the drug, while the epidemiological studies cannot by their nature prove that the drug is in no sense teratogenic — merely that such effects as there may be are undetectable against the background.

So where is the difficulty? In such circumstances, there is no reason why the parents of a malformed child should not sue on his or her behalf the manufacturers of the drug for damages. The case may not be as clear cut as with the now admittedly harmful effects of thalidomide, but at least it is possible for a lawyer to make a case. So far, in the United States, two such suits have come to trial. One has been lost, in the other a jury has recommended the award of damages but the trial judge has yet to decide whether to confirm that or to reach some other verdict. Meanwhile, a further 300 cases are pending, while the use of the drug has steadily declined — British prescription sales are a quarter of what they were five years ago — as publicity has persuaded pregnant women that the discomforts of morning sickness are preferable to the risks of taking the drug, however small they might be. In the circumstances, the decision to stop production of the drug is probably commercially prudent as well as, more generally, politic. Even so, the decision will be remembered as a landmark of a kind — one of those occasions when the licensing approval of the Food and Drug Administration (in the United States), the Committee on the Safety of Medicines (in the United Kingdom) and similar bodies elsewhere has counted for less than the groundswell of public opinion. Unreason seems to have won the day.

The dangers in this development should not be overlooked. Whatever the truth about Debendox and its equivalent elsewhere, the facts are that it had been approved by the regulatory authorities in several countries and also found useful by physicians. So there can be no complaint against the manufacturers for having put it on the market. Yet as things have turned out, the manufacturers must now write off the cost of their past investment in the drug and also shoulder that of fighting the legal suits still pending. But, it will be argued, the Dow Chemical Co. Inc., the sole shareholder in the various Merrell enterprises, is rich enough to bear the cost. That is a shortsighted argument. First, nobody should be surprised if the costs are in the long run borne by the company's other pharmaceutical products, which is perfectly legitimate in all countries where the institution of the joint-stock company is respected. Second, what would have happened if the manufacturer had been a small and not a large company, unable to shoulder the costs without going bankrupt? Sheer equity requires that companies that behave responsibly should not be driven to the wall for such reasons. What remedies

may there be? One obvious course is to arrange that the approval of the licensing authorities can to some degree be pleaded in aid by manufacturers plagued by vexatious suits. In logic, of course, if some manufacturer's drug has been approved, faults that afterwards come to light should be laid at the door of the regulatory authority, not the manufacturer. Governments will not wear that on the proper grounds that their regulatory bodies are not equipped physically to carry out investigations of new drugs and that a responsible manufacturer has a continuing duty to carry out the research required to show that the ultimate users come to no harm. But the regulatory bodies cannot remain entirely aloof if a product they have licensed should afterwards be challenged. At the very least, they might be expected by the courts to say why they had licensed the drug in the first place, and to give an opinion on the adequacy of a manufacturers' handling of the material after it had been put on the market.

The present assumption that these bodies have no responsibility is too po-faced for the real world — and is a threat to other potential therapeutic agents. And the case of Debendox is not the first on which public uneasiness about the side-effects of a new drug has limited its practical usefulness. The present use of whooping cough (pertussis) vaccine in Britain, for example, is less than it might be because of a spate of public anxiety about untoward side-effects — with the result that there was a mercifully minor flare-up of the infection earlier this year. Nobody suggests that the regulatory agencies should become protagonists for the pharmaceutical industry, but to the extent that their existence stems from people's wish to have access to drugs that are effective and also safe, they have a residual responsibility for what they wish on the public. If, on some occasions, they and not the manufacturer of a new drug are held to share part of the blame for unexpected side-effects, they should be provided with the funds to pay what the courts consider to be their share. □

Polish precedent

The Polish Government, in rooting out dissent, may be the chief loser.

THE Polish authorities appear to have found a novel way of dealing with unwilling and recalcitrant members of public laboratories — split the laboratory into separate units, each of which is called by a different name, and then invite staff members from the original institution to reapply for what are in essence the jobs they held previously. This is how the Institute of Nuclear Research in Warsaw, thought to have been sympathetic to Solidarity before December 1981, has been brought to heel in the past few months. The institute itself, one of the most distinguished in Poland, has been split up and the process by which the members of its staff have been accredited anew to its component parts has tipped many of them out. Meanwhile, the theoretical division of the institute has been left in limbo. Its members' contracts expire at the end of this month, but there are as yet no places for them in the new institutes. Moreover, those concerned do not know whether the government intends to tip them out into the streets or whether, alternatively, it is simply playing cat and mouse.

This is a shabby way to deal with professional people, whatever



their political inclinations and sympathies. Moreover, it is as demeaning for the Polish Government as for those who do not know whether they will have jobs to go to next month, for the institute that has been broken up is one of Poland's links with a distinguished tradition. Leopold Infeld, Poland's best known theoretical physicist, who ran an institute at the University of Warsaw, was an important influence in the founding of the Institute of Nuclear Research — indeed, his own university group and that of the nuclear research institute lived in each others' pockets (and in the same building). If the Polish Government now puts the theoretical physics division to sleep, it will find that the procedural benefits of a compliant research force are outweighed by the intellectual damage done to its research enterprise — and the damage suffered by its reputation elsewhere. □

Arms control by shouting

The two superpowers should learn to be less categorical about their objectives in arms control.

THE two superpowers, the United States and the Soviet Union, appear equally determined to ensure that the arms control negotiations in Geneva will run into the sand by their insistence on negotiation by means of public declarations. That is one interpretation, admittedly sinister, of the way in which the two governments have conducted themselves in the past few months. The negotiations on missiles of intermediate (or European) range were hamstrung until earlier this year by the assumption that President Reagan's year-old "zero-option" (no US cruise missiles or Pershing II rockets in Europe, no Soviet SS20s within range of Europe) was the US Administration's last word on the subject, an assumption that Mr Reagan and his colleagues quickly came to share. The result, earlier this year, was that Western European governments spent much energy persuading the United States to be more flexible, a point eventually conceded but only against the noise of repeated Soviet declarations that the British and French nuclear forces must be counted in the same negotiations and, last week, that the planned deployment of new missiles in Western Europe could well be matched by the siting of SS20s in the Warsaw Pact countries to the west of the Soviet Union.

A closely similar sequence of declarations and counter-declarations seems in prospect in connection with the Strategic Arms Reduction Talks (START), resumed last week in Geneva. President Reagan announced that the United States would be flexible about the numbers of missiles allowed each side under some future treaty, and that it will insist only that the numbers of strategic warheads should be limited (to 5,000 apiece), with the necessarily vague proviso that there should also be a limit on the throw-weight (effective payload mass) of the two missile forces. Wisely, on this occasion, President Reagan emphasized that he has told his negotiators to be flexible.

High time, it will be thought. The snag, of course, is that the mere acknowledgement that the throw-weight issue is, for the time being, an important part of the US negotiating position gives a hostage to fortune. The lifting capacity of Soviet rockets is known to be greater than that of those in the United States, but their destructive potential is not necessarily larger. The consequence is that the Soviet Union is likely to jib at what the President is proposing and that, if there is eventually a draft agreement to consider, there is certain to be a powerful demand from Congress to know why the administration has abandoned an objective considered important in mid-1983. Nobody asks that people like the US President and his opposite numbers in the Soviet Union should keep silent about these missile negotiations — indeed, there are powerful reasons why they should assure their constituents at home and overseas that they are serious about the two most important sets of arms control negotiations now under way. But there are the strongest possible reasons why they should not be thought to be committed to positions that in the end may be unattainable but which, having become part of the public understanding, may frustrate agreement. To say this is merely to restate what diplomacy is about. □

Let there be trade

This year's UNCTAD meeting in Belgrade is an opportunity for both rich and poor countries.

WITH a little luck, this month's meeting of the United Nations Conference on Trade and Development (UNCTAD) at Belgrade should be more constructive than its predecessors. Past form has been depressing. Usually, meetings originally intended to encourage trade in the commodities that are the chief source of export earnings by developing countries have instead been used as opportunities for the developing countries to upbraid their richer colleagues about the inequity of the present economic system as a whole. Although nobody can fairly claim that relations between the rich and poor nations can be entirely divorced from politics, no worthwhile purpose can be served by raising questions that UNCTAD itself is not competent to deal with. Now, however, both sides in what has been a long-standing wrangle — UNCTAD conferences happen every four years — have different incentives to talk sensibly to each other.

First, rich countries know more clearly than ever that their own economic well-being will be damaged if important developing countries are forced by penury to default on their loans from the international banking system, and so appreciate what the second instalment of the Brandt report urged earlier this year — that the encouragement of exports from developing countries is an urgent need. The developing countries, on the other hand, appreciate that their problems, always serious, are now too desperate to be subsumed in rhetoric. A new world economic order would be a fine thing to have if there were time to agree what it might consist of. In reality, the danger that the international economic system might collapse like a house of cards is too real for the comfort to be attainable. The time has come for plain talk.

The starting point should be the recognition that international trade is not a zero-sum game. The more one country is able to export, the more it can afford to import, with the result that its trading partners' exports are increased, their capacity to import in turn augmented and so on until everybody feels a little more prosperous. What has been happening in the past ten years, however, is that the feedback has been negative, not positive. The deflationary consequences of increased oil prices have been twofold — the encouragement of protectionism among groups of well-heeled countries (which has helped to reduce the export of rudimentary manufactures and agricultural products from the developing countries) and the general reduction of economic activity, which has depressed the market prices of primary commodities on which many developing countries depend. The result is that international debts incurred in the heady 1970s, when trade was forever on the up and up (or when, as in the case of Mexico, oil prices seemed destined to remain high), have now become millstones round the necks of all but the poorest countries — those so badly off that their credit could not command big loans. The problems with which many of the poorest countries must contend have been further exacerbated by their own mismanagement of their affairs, by their perverse neglect of agriculture in particular.

The standard remedy for this perennial problem is to argue that the volume of international credit should be increased. There is indeed a proposal by the International Monetary Fund that this should be done, although it is unlikely that the extra funds (in the form of Special Drawing Rights) will be available before the early months of next year. But extra credit will do little to help countries whose economies are internationally bankrupt to begin with. What the poor countries need, in any case, is trade, not credit. UNCTAD should therefore look at more courageous developments. The international banking system should be prepared to take forward contracts for the delivery of commodities as a substitute for interest payments now due. And protectionist rich nations should agree among themselves on a policy to liberate international trade in agricultural commodities, putting up with a modicum of domestic dislocation for the promise of more sophisticated trade to follow. ||

NIH budget

Political control a high price to pay for funds?

Washington

CONGRESS'S annual habit of adding more money to the budgets of the National Institutes of Health (NIH) than the White House tries to take out looks like being a mixed blessing this year. Fears are growing within NIH that if Congress is too generous it will simply invite a presidential veto. And there is outright alarm at the prospect of Congress coupling a big spending increase to a package of radical changes in the agency's management and structure.

A reauthorization bill designed to do just that is expected to receive the blessing of the House of Representatives later this month. Proposed by California Democrat Henry Waxman, the bill (HR 2350) would increase NIH spending in some areas in 1984 by 15 per cent instead of the 1.8 per cent proposed by the administration. It would also transform the internal management of NIH, replace its present broad budget authority with a set of detailed congressional mandates, and establish a new institute for arthritis and musculoskeletal diseases.

The bill is being opposed with equal ferocity by the Office of Management and Budget (OMB) and NIH. OMB has written to House minority leader Robert Michel warning that the bill will almost certainly be vetoed by President Reagan. And Margaret Heckler, the Secretary for Health, has told Congress that the bill would disrupt the orderly management of NIH and lead to political control of scientific decisions.

Waxman maintains that the existing statutory authority for NIH is too general. Changes proposed in the Waxman bill range from major institutional realignments — such as the transfer to NIH of the National Institute for Occupational Safety and Health, currently part of the Centers for Disease Control — to relatively minor procedural changes such as a new requirement that NIH submit a biennial report to Congress. Almost every change has been criticized by NIH's leadership.

In hearings staged by Waxman's health and environment subcommittee earlier this year, NIH contended that creation of a new arthritis institute would waste money that could otherwise be used for research, by creating unnecessary administrative costs. NIH director James Wyngaarden said spending on arthritis and musculoskeletal diseases was already one of the fastest growing areas in NIH research, with spending up from \$27 million in 1976 to some \$83 million in 1983.

NIH also object to a provision in the bill that would compel each institute to appoint an assistant director for disease prevention

and establish, within three years, 25 health promotion and disease prevention centres each costing up to a million dollars every year. Dr Edward Brandt, assistant secretary for health, told the Waxman committee that prevention of disease was the ultimate goal of all NIH research and that the link between research on prevention and the institute's basic scientific programme should remain unbroken.

Other new authorizations and budget set-asides in the bill are new initiatives in bioengineering, digestive disease, kidney disease and mental retardation. There are to be new grants for specific research in spinal cord regeneration, Alzheimer's disease and animal research methods. New national commissions on neglected diseases and genetic engineering are proposed.

Detailed organizational changes called for in the bill include a controversial proposal to enhance the powers of advisory committees within NIH. For the first time, the functions and composition of the committees would be enshrined in statute and they would be called on to review the quality of intramural research at NIH, as well as to advise on the allocation of space and money between individual laboratories and

investigators. The administration has described as "deeply troubling" a proposal to expand the scope of the director's advisory committee to allow it to embrace management issues such as the acquisition of resources and the hiring of personnel.

The criticism from NIH and the administration is not expected to prevent the bill from receiving enthusiastic support when it reaches the floor of the House. The big increases it proposes for cancer and heart disease research are traditional vote-winners in the Democrat-controlled chamber, and the bill caters for numerous special interest groups concerned about issues such as the use of animals in research and the ethical implications of genetic engineering.

There is, however, a substantial minority of opponents in the House. Eleven members of the Energy and Commerce Committee, which has just reported out the bill, added a minority report complaining that the excessive detail of the authorizations represented an attempt by Congress to take the place of scientific peer review in determining priorities in research. The dissenters pointed out that the bill would pre-empt the efforts of the National Academy's Institute of Medicine, which is beginning a wide-ranging study of NIH's organizational structure.

At NIH, meanwhile, officials are pinning their hopes for scotching the bill on the willingness of the President to veto a measure which will inevitably command extensive public support. **Peter David**

Cuts "disastrous" for Canada's MRC

Washington

CANADIAN medical researchers have since the beginning of this year been quietly lobbying the government to rescind a substantial cut in the budget for the Medical Research Council (MRC) for the fiscal year beginning 1 July. Two weeks ago, eight prominent Canadian scientists abandoned the quiet approach with a sharply worded protest that calls the cuts "disastrous" and warns of "lasting damaging consequences".

MRC's problems stem from a government decision early this year to limit the "social development" ministries, the group that includes MRC, to a 6 per cent increase in the next fiscal year. According to Dr John Cowan, chairman of the University of Ottawa physiology department and one of eight former presidents of the Canadian Federation of Biological Societies who signed the statement, inflation in medical research costs has been running at 18 per cent. The statement says that when MRC met in March to award research grants for the next fiscal year, it was able to fund only 10 per cent of the proposals received — down from 30-35 per cent in previous years. Some 80 per cent of the proposals were recommended for support, according to the statement.

Similarly, renewals of existing grants were down; fewer than half the usual number of scholarships were awarded; and no major equipment purchasers were approved, nor were even minor equipment purchases included within research proposals. Only "very few" equipment maintenance grants were approved.

Under the Canadian budget process, a supplemental budget for a specific area can be approved by the cabinet at any time, although this is usually done before the start of the fiscal year. Cowan said that the Natural Sciences and Engineering Research Council, MRC's counterpart, had received a supplementary budget giving it a 20 per cent increase over the current year. The medical researchers' statement asks for a \$20 million supplement for MRC, which, the researchers say would allow MRC to continue to operate at its previous levels, and would be in line with the increase received by the natural sciences council.

Cowan said that he and others had waited until now to act because the medical research community had been "persuaded by the MRC and the federation itself not to rock the boat" while the 5-year planning document for MRC was awaiting a cabinet decision. **Stephen Budiansky**

High-energy physics

Another flavour of quark?

HARD on the heels of the discovery of the Z^0 particle by the UA1 group at CERN (European Organization for Nuclear Research), rumours were rife that the group has also discovered the "top" quark — the sixth flavour in the family. This development has not been confirmed, however. The position seems to be that among its harvest of possible $W^\pm$ and Z^0 events, the UA1 group has a few events that are "consistent with" the existence of top. The difficulty is that, hitherto, the criteria used for selecting for analysis records of proton-antiproton collisions have been biased towards putative heavy-meson events. Nevertheless, the data to sustain a search for the top quark exist, but the search will not be started until the data on the W s and Z^0 have been tidied away.

Meanwhile, the UA1 group is rushing out a paper on the intermediate vector bosons giving the latest estimates of their masses as follows:

	UA1 estimate	Salam-Weinberg theory
Z	95.2 ± 2.5	94 ± 2.5
W	81 ± 2	83 ± 3

The measurements are clearly consistent with the theory. Moreover, the UA1 group has also measured the "Weinberg angle" θ_W , an essential parameter of the unified theory of electromagnetism and the weak nuclear interaction. The value quoted in the paper from the UA1 group is $\sin^2 \theta_W = 0.226 \pm 0.011$, again consistent with earlier estimates of 0.236 ± 0.030 .

Meanwhile, CERN has had success of another kind. The French Government at the end of last month gave its formal blessing to the construction of LEP, the large electron-positron collider due to be completed by 1989. Armed with the government's blessing (a *declaration d'utilité publique*, an environmental requirement for big constructions), CERN is now purchasing the land it requires around the ring for surface buildings.

There has been strong local opposition to these buildings, particularly in the village of Echevex — where villagers claim the buildings are far too close and noisy. The opposition to LEP now has no further legal line of resistance, but the legal owners of the land could have some influence by refusing to sell at the price CERN offers, when a judge would have to arbitrate. CERN officials say, however, that the farmers who own the land at Echevex are accepting the offers. Construction is now expected to begin in July, with injection of electrons and positrons beginning in late 1988 and first experiments in mid-1989.

The pace of development may, however, be affected by the interests of the UA1 physicists and others in improving the antiproton-proton collider which has pro-

duced the Z s, W s and possibly top. There is a proposal for a SF50 million (£15 million) development of the collider to improve its luminosity (production rate of events such as W s and Z s) tenfold. This would take three years and could thus be just ahead of Fermilab (see below). The snag is that the member governments of CERN are not flush with cash just now.

Even so, Dr Erwin Gabathuler, CERN's director for colliding beams, is hopeful. He argues that member states should acknowledge that the collider has wrested the lead

US particle accelerators

Fermilab poised to leapfrog

Chicago

THE Fermi National Accelerator Laboratory is jubilant over its achievement of a long-sought goal, the operation of the world's first large superconducting particle accelerator. The machine is called the energy doubler/saver, for its twin goals of doubling the maximum energy of Fermilab's proton beams to 1,000 GeV — one teravolt — and of using electrical power more efficiently. For several years, Fermilab has been able to afford power for its accelerator for only about half the year.

Beams in the superconducting ring, also called the Tevatron, were circulated at 100 GeV (1 GeV = 10^9 electron volts) for the first time this month. Experiments with the new beam are scheduled for October at 400 GeV, and beams approaching 1 TeV are expected early next year. Development of the superconducting accelerator began at Fermilab in 1972, while the conventional 400 GeV accelerator was still being broken in. The machine was the idea of Fermilab's builder and first director, Robert R. Wilson, who reasoned that the cheapest way to increase that accelerator's energy was to build a new ring of powerful superconducting magnets in the same tunnel.

The technology of superconducting magnets was not then as advanced as some had hoped. The US Government complicated matters by reducing funds for high-energy physics, which prompted Wilson's resignation in 1977. While Fermilab and another American accelerator projector, Brookhaven Laboratory's ISABELLE, were bogged down on the frontier of superconductivity, the European Organization for Nuclear Research (CERN) moved in 1979 into the different technology of colliding beams of protons and antiprotons, making available much more energy in inter-particle collisions than can be had from fixed targets.

With the 1,000 GeV Tevatron beam, only 45 GeV will be available to create new particles, far less than the 540 GeV available from CERN's 270 GeV beam, which has made possible the recent

in high-energy physics from the United States. He also says that the net cost of SF50 million could be brought down to SF20–30 million by using some of the contribution from the new member, Spain (which wished to support mostly lower energy physics at CERN). And the money would be spread over three years, he pleads.

But members are more likely to require that if the collider is to be improved, it should be within a constant CERN budget. That might entail a delay in the LEP programme — which is itself in competition with an American proposal, the SLAC linear collider. The ball thus seems likely to end up right back in the physicists' court.

Robert Walgate

discovery of the W and Z particles. Fermilab is now building the antiproton accumulator and other machinery necessary to use the Tevatron as a collider, but it is not expected to operate until 1985–86.

According to Chris Quigg, head of the theory group at Fermilab, intense beams of secondary particles such as neutrinos and muons produced by the Tevatron will be ideally suited to study the quark structure of nucleons. The fixed-target accelerator will also be unsurpassed for studying short-lived particles that contain charmed and bottom quarks. The tau neutrino, expected as a companion of the tau lepton, is also a candidate for discovery.

But Quigg says that qualitatively new physics is expected to appear at Fermilab in 1985, when the Tevatron is first used as a collider. If all goes as planned, the machine will produce collisions four times as energetic as CERN's and with as much as ten times the luminosity. One prize in contention between Fermilab and CERN will be the top quark, the sixth and perhaps the last quark flavour (see above), as well as a determination of the total number of neutrino species in the Universe (by measurements of the energy width of the Z) and the mechanism of the rare Centauro events hitherto seen only in cosmic rays.

Of CERN's recent successes, Quigg says, "The European strength in high-energy physics is healthy for the field as long as we keep up our end of it. In two years it will be our turn again. As long as we keep alternating the big new machines this way, the competition between CERN and Fermilab will be a good thing". By 1986, Fermilab's 2TeV collider will be the most powerful of its kind". But in 1989, CERN hopes to commission LEP, the most powerful electron-positron accelerator. Whether the United States will then be able to look forward to its next turn depends in large part on the recommendations to be made this month by physicists meeting at Woods Hole, Massachusetts, to discuss the next generation of US accelerators.

Larry Arbeiter

Gene manipulation

Churches against germ changes

Washington

A RESOLUTION calling for an immediate ban on human genetic engineering that alters germ cells was released last week over the signatures of 21 Catholic bishops, a broad spectrum of Protestant and Jewish religious leaders and three scientists — George Wald and Ruth Hubbard of Harvard University and Ethan Signer of Massachusetts Institute of Technology.

The resolution cites "new advances in genetic engineering technology" that "raise the possibility of altering the human species", and concludes that humans have no right to decide which genetic traits should be introduced and which eliminated from the human gene pool.

The statement appeared to take a much harder line than that espoused by religious leaders and theologians who testified before the President's Commission on Bioethics, which last year produced a report on human genetic engineering called *Splicing Life*. Hearings on the report called in November by Representative Al Gore (Democrat, Tennessee) elicited strong support for the commission's approach of increased scrutiny together with no *a priori* bans on human genetic engineering. Several clergymen who testified before Gore's subcommittee also signed last week's resolution.

Morris Abram, chairman of the now-defunct President's Commission, expressed puzzlement at the resolution. "These techniques have already demonstrated a great potential for relief of suffering and possible diagnosis and treatment of diseases", he said. He added that human genetic engineering does indeed raise ethical problems, but that other medical procedures, such as artificial insemination and even cancer chemotherapy, raise similar problems. Alex Capron, former staff director for the commission, was more outspoken. Calling the resolution "knuckleheaded", he complained that it swept aside difficult ethical issues in favour of a "broadside attack".

Several of the signers were quick to explain that they did not fully agree with the resolution nor with a supporting letter that attempted to explain its philosophical basis. Both were written by Jeremy Rifkin, an author of popular books on social issues. His latest book, *Algeny*, was recently published, and this has prompted critics to question the timing of the resolution.

Father Richard McCormick, SJ, of Georgetown University's Kennedy Institute for Ethics — a witness at the Gore hearings — explained that he did not actually agree with the resolution's call for a ban on germ-cell engineering, but had signed it as a "good vehicle for public discussion" of the issue.

Other signers stressed that they did not object to genetic engineering of human

somatic cells. James Malone, a Catholic bishop and vice-president of the US Catholic Conference, issued a statement to that effect, noting that it was "in line with papal teaching"; Pope John Paul II last year told scientists assembled at the Pontifical Academy of Sciences of his "great hope" that genetic engineering could help those afflicted by genetic diseases.

But, as Capron noted, it is probably premature to be taking stands on either somatic or germ-cell engineering in humans. Although alteration of germ cells has been carried out in mice in one admittedly crude experiment (see *Nature* 300, 811; 1982) the only attempt to alter genes in humans — Martin Cline's unauthorized implantation of genes coding for red blood cells in the bone marrow cells of two women with a fatal genetic disorder, an attempt to alter somatic cells — was a dismal failure and a professional disgrace.

If these techniques do become prac-

ticable, however, a restriction of their use to somatic cells would probably rule out effective gene therapy for genetic diseases such as cystic fibrosis, which affect every organ of the body. Even diseases such as diabetes, which affect one organ, could probably be attacked more directly through the germ cells or the zygote.

According to Rifkin and some of the resolution's signers, the danger is that no line can be drawn between eliminating a genetic defect from the human gene pool and eliminating merely undesirable traits. "Indeed, what is to preclude society from deciding that a certain skin color is a disorder?" Rifkin writes in the letter accompanying the resolution.

Capron counters that the "supreme irony" in Rifkin's resolution is that by calling for government regulation in deciding what is acceptable human genetic engineering and what is not, the very danger that Rifkin is warning of — eugenics — is made all the more likely. The government, Capron says, would then be left with the responsibility of deciding which diseases gene therapy could be applied to.

Stephen Budiansky

Data falsification

Innocents and culprits

Washington

ONE of the two committees set up to investigate the noted work of Dr Karl Illmensee of the University of Geneva announced last week that it had found no evidence of fraud. Following charges by members of his own laboratory of irregularities in Illmensee's experimental work (see *Nature* 2 June, p.363), the University of Geneva and the Jackson Laboratory in Bar Harbor, Maine, each appointed external committees to investigate the work. The Bar Harbor committee, chaired by Dr Dorothea Bennett of the Sloan-Kettering Institute for Cancer Research, "searched for evidence of possible fraud and found none". The committee did suggest however, that Illmensee and his collaborator, Dr Peter Hoppe, should repeat the experiments using an experimental design that will yield "unequivocal" results. Meanwhile, the University of Geneva has still not finalized the membership of the international committee of inquiry that is to investigate Illmensee's work in Geneva.

Dr Wilbert Aronow, a prominent cardiologist who last year agreed to disqualify himself from new drug trials amid evidence that he falsified data (see *Nature* 14 April, p.560), was fired last week from Creighton University after an Environmental Protection Agency (EPA) investigation found that studies he had performed for EPA on carbon monoxide were similarly flawed.

The EPA investigators reported "considerable questions" about the validity of Aronow's studies, which have played a critical part in setting the ambient air quality standards for carbon monoxide.

Although the investigators said they "could not resolve the issue of possible falsification of data", they found that raw data had been discarded or lost, adequate records had not been maintained, and experimental controls were poor or non-existent. The only raw data that Aronow was able to produce were from the most recent of 10 carbon monoxide studies Aronow had carried out since 1971. Interviews revealed that no log books had been kept during the experiments and that much of the data had been recorded on "bits of paper" and "looseleaf notepaper".

A revision of the carbon monoxide standard has been pending since 1980. Some EPA officials had wanted to loosen the standard, but Aronow's data — which show adverse health effects of very low levels of carbon monoxide — convinced the agency that it should keep to the 1980 proposal, which differs very little from the standard currently in effect. (The current standard sets a limit of 9 carbon monoxide over an eight-hour average; the proposed revision would allow localities to exceed that limit once a year.)

The EPA investigation, headed by Dr Steven Horvath of the University of California, Santa Barbara, began in April after EPA officials read press accounts of Aronow's voluntary disqualification by the Food and Drug Administration (FDA).

Aronow resigned as chief of the cardiovascular section at the Veterans Administration hospital in Long Beach, California, last summer during the FDA's investigation of his drug trial data. He has been on a one-year appointment at Creighton since then. Stephen Budiansky

Space Telescope

Prospects bright on ground

Baltimore

THE well-publicized setbacks in the management and construction of the Space Telescope (see *Nature* 2 June, p.366) seem to have left few scars on those working at the Space Telescope Institute, whose new building will be officially opened this week. Sited on the bank of a meandering stream in a leafy corner of the Johns Hopkins University Baltimore campus, the calm interior of the already filled building seems consistent with the view of the institute's senior staff that their problems have been relatively minor, and will not contribute significantly to delay or extra costs experienced by the Space Telescope mission.

The main problems that face the Space Telescope have arisen principally within Perkin-Elmer, the subcontractor responsible for much of the hardware. The much lauded 2.4-m mirror constructed by Perkin-Elmer has become contaminated within the company's "clean room", significantly decreasing its reflectivity. Although no specific plans have been agreed, everyone expects that the mirror will be cleaned before launch. Another significant problem concerns the fine guidance sensors, interferometric devices giving the telescope the superb positional accuracy which its refined optics require. Problems in the development of these devices at Perkin-Elmer seem likely to delay the project by a year (to late in 1986) and to add \$100 million to the total cost.

Neither of these problems is the responsibility of the Space Telescope Institute, whose staff expects that the performance of the Space Telescope should be undiminished. Broadly speaking, the job of the institute's director, Riccardo Giacconi, and his staff will be to maintain and schedule the telescope's operation, to coordinate the retrieval and storage of the data from the telescope's six detectors and to administer access by astronomers.

One task nearing completion is the development of the Guide Star Selection System (GSSS). The Fine Guidance System has to scan over the 69 arc-min² field of view to locate previously programmed stars to guide the telescope. It is hoped that the telescope's pointing error will be as small as 0.007 arc-s. To ensure the presence of usable stars for any orientation, a photographic plate library down to visual magnitude 14.5 (2,400 times fainter than can be seen with the naked eye) has been compiled. By next September, it is expected that the institute's two scanning microdensitometers and their associated VAX 11/750 computers will have been programmed so that the guidance star catalogue can be drawn up before launch.

The total remaining computing hardware at the disposal of the Space Telescope is formidable, including six linked VAX computers (two of which are at the NASA

Goddard Space Flight Center (GSFC) through which all communication with the Space Telescope will be channelled) and ten image processing systems for real time support and for data analysis. The "Science Operations Ground System" which is to incorporate these devices has nevertheless been something of a headache for the institute's personnel.

The original specifications were drawn up by GSFC, with scientific advice, and carried out by TRW Systems Inc. The institute has spent more time than anticipated on revising those initial plans. Adrienne Timothy, the institute's project manager, explained that it might have been better if the institute had had a year's more lead time so that the system's development could have been better coordinated.

Similar problems have arisen in the institute's instrument support branch. The detectors fixed to the Space Telescope's focal plane will include a high speed photo-

meter, faint object camera, wide field/planetary camera, faint object spectrograph and high resolution spectrograph, each of which is being developed elsewhere.

None of these problems appears to threaten the programme. Indeed, Adrienne Timothy described them as the teething problems one might expect in a project of this magnitude. Nevertheless, she agreed that the solution to these problems had been achieved partly to the detriment of the institute's research staff. This includes 40 or so astronomers, mostly experienced workers with established reputations who had expected to spend half their time on research and have instead been coping with the project's teething problems. Negotiations are now under way with NASA and subcontractors in the hope that these difficulties can be alleviated. Certainly the institute's official policy is that, by launch time, the staff should spend half their time on research. Thereafter, most of them will have to compete with the rest of the community for observing time, the peer review process being conducted outside the institute. **Philip Campbell**

UK-Dutch astronomy

Mauna Kea telescope in view

CONSTRUCTION has started on the largely British-Dutch 15-metre millimetre-wave telescope on Mauna Kea, Hawaii. The telescope, costing £7 million, is to be financed 80 per cent by the UK Science and Engineering Research Council and 20 per cent by the Netherlands Organization for the Advancement of Pure Science. The observatory will be at an altitude of about 14,000 ft — well above most of the water vapour that impedes millimetric observations at lower altitudes — and is expected to be completed by 1986. The Royal Observatory, Edinburgh, already responsible for the UK Infrared Telescope at the same site, will administer the facility, the combined running costs being £1.5 million a year.

Two factors apart from atmospheric water vapour have hitherto hindered the development of millimetre-wave telescopes. One has been the lack of sufficiently sensitive receivers, but the recent development of very low temperature detectors with elements only one micrometre square has alleviated such problems. The other factor was the accuracy needed in the construction of the paraboloid reflector surface. Mechanical and thermal distortions should not distort the surface beyond a small fraction of the operating wavelength — in this case, 50 micrometres. These problems should be overcome by the lightweight aluminium honeycomb structure and the thermal protection provided by the telescope's enclosure. The latter incorporates a membrane which will reflect most visible and thermal radiation but which will transmit millimetre radiation.

The new telescope is of particular significance because of the poor state of health

of millimetre astronomy in the United States, where plans for a 15-metre millimetre-wavelength telescope collapsed last year. Apart from initial proposals for a new design study for an aperture synthesis array (see *Nature* 5 May, p.7), there are no comparable US facilities in sight.

A hint of the potential benefits came a few weeks ago, when Japanese astronomers reported the detection of a rotating gas cloud surrounding a massive proto-star in Orion. Using the Nobeyama 45-m dish, they observed the system at a wavelength of 6.1 mm (a spectral wavelength of carbon monosulphide). Dr N. Kaifu and his collaborators reported that the system contained a central proto-star of about 50 solar masses surrounded by a gaseous disk of 200 solar masses, rotating and gradually contracting from a radius of 40,000 AU (just over half a light-year). However, the presence of a strong solar wind would, said Dr Kaifu, probably prevent the formation of planets.

To some extent, the British and Japanese telescopes will be complementary. While the Japanese telescope is much larger in diameter, the accuracy of its surface paraboloid is to within about 0.2 mm, compared with the 1/40 mm for the British-Dutch telescope. Thus the latter will be able to observe with an astronomical resolution of 10 arc-s resolution at only 1/3 mm wavelength. The Japanese may reach comparable resolution, but at longer wavelengths. In 1985, however, they will have a 5 × 10 m dish interferometer with which they hope to observe the CO line (2.6 mm wavelength) with an accuracy of 1 arc-s.

Philip Campbell & Robert Walgate

Polish nuclear physics

Future of institute in doubt

THE future of theoretical nuclear physics in Poland is in some doubt, following the closure last December of the nuclear research institute at Swierk (see *Nature* **301**, 103; 1983). The original institute is to be replaced by three: the Institute of Atomic Energy, the Institute of Chemistry and Nuclear Technology and the Institute of Nuclear Problems. The research programmes and organization of these new institutes are now being worked out. Nothing, so far, has been said about the future of the former Theoretical Physics Division at Swierk.

The reorganization of such an important institute as Swierk is widely believed to have been a cover for a purge of Solidarity activities. Swierk had, for many years, been a focus of dissent — in 1976, two young Swierk scientists, Grzegorz Boguta and Mirosław Chojecki, joined the Workers' Defence Committee (KOR), the civil rights pressure group that paved the way for Solidarity.

In the summer of 1981 when, after an interval of five years, the Polish Government reestablished its Atomic Energy Agency and put it in charge of Swierk, the scientific council of Solidarity at the institute refused to accept the introduction of "Deputy Directors for Security" who would be appointed from the state security apparatus. On the imposition of martial law, Solidarity members at Swierk went on strike, in spite of the new regulations outlawing strike action.

More than 70 Swierk employees were interned, arrested, fined or dismissed. Professor A. Wierusz, director of the nuclear power development programme, and Dr Zygmunt Luczynski, a specialist on gas-phase ion-molecule reactions, were interned and Dr Tadeusz Pacuska, a leading biochemist, received a two-year prison sentence for participating in a strike. Others were repeatedly interrogated by the police, and were refused passports to attend conferences abroad.

The formal closure of the institute last December meant that even if a researcher expected to continue doing the same job in the same laboratory under the new set-up, he or she had to go through the political verification process imposed on new employees. It also provided a loophole for sacking researchers who had held tenured positions for many years. At least 40 people lost their jobs in this way, including Dr Wierusz and Dr Luczynski (by now out of internment), Dr J. Kownacki (a specialist on heavy nuclei), Dr T. Krogulski (muonic atoms), Dr E. Piasecki (fission) and Dr Zenon Wegrzynowicz (a biologist).

According to the Solidarity underground in Warsaw, the proton synchrotron team was particularly hard-hit.

Moreover, instead of being allowed to work out their statutory three months'

notice, the dismissed scientists, contrary to Polish law, were not allowed to return to the institute while under notice. They had to collect their salaries and ration cards outside the institute premises, and their experiments were simply abandoned. The dismissals did not go unmarked by the Polish physics community, and resolutions expressing concern at the manner in which they were carried out have been passed by both the University of Warsaw physics department and the Physics Committee of the Polish Academy of Sciences.

At the end of March 1982, Grzegorz Boguta, the former member of KOR, was refused an extension to his contract, although a position was available within the new structure. This meant that he would be unable to complete his PhD research in radiobiology. A petition on his behalf was signed by 46 fellow employees in the department of radiobiology (only 3 abstained) but was rejected by the authorities. A few days later, Dr Maria Kopec, his supervisor, who had been particularly active on his behalf, was demoted from her position as head of the depart-

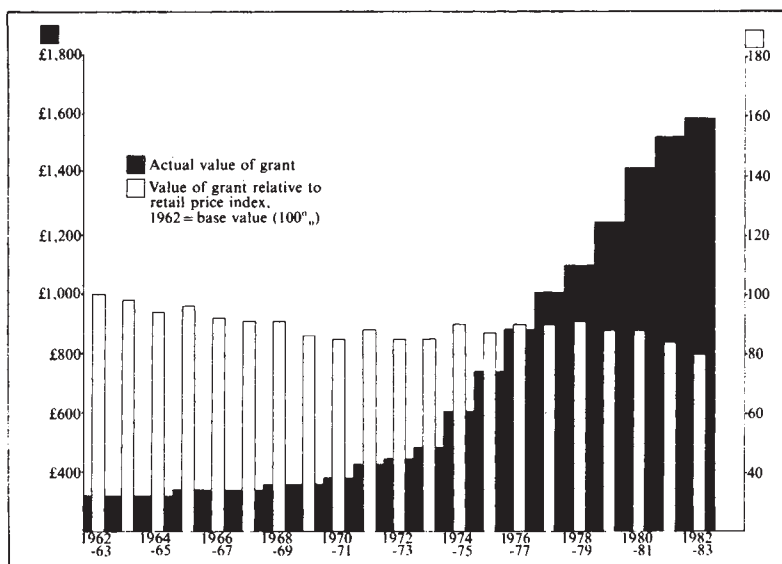
ment and has now been forced to take early retirement.

Ironically, and almost simultaneously, the Polish Radiation Research Society awarded its Maria Skłodowska-Curie medal to Dr Kopec and a special prize to Mr Boguta, and elected Dr Luczynski to its executive board. A few days later, the military commissar for the Swierk complex ordered an investigation into those taking part in the society's elections. The latest rumours suggest that another round of mass dismissals from Swierk is on its way.

Under these circumstances, the outlook of the theoretical physics division looks bleak. Its research teams include such internationally known figures as Dr E. Infeld (the son of Leopold Infeld, one of the founders of Swierk and a colleague of Einstein), Professor R. Raczka (field theory), Professor J. Dabrowski (field theory) and Dr L. Lukaszuk (particle theory). Formally, the decision as to whether to disband the division or to incorporate it into the new structure is the responsibility of Dr M. Sowinski, the director of the Atomic Energy Agency, although it is hoped that Professor M. Nalecz, who holds a watching brief within the agency on behalf of the Academy of Sciences, will be consulted.

Vera Rich

Changes in UK students' grants



THE recent announcement by the British Government of a 4 per cent rise in the undergraduate student grant has met with widespread discontent, particularly within the National Union of Students (NUS). The undergraduate grant is a mandatory award given to permanent residents of the United Kingdom who are undertaking "designated" further education courses, which include, for example, first degree and teacher training courses. The grant is subject to a parental means test and the dispute is centred around the relative value of the maximum grant which may be awarded to students in England and Wales. The

grant for all parts of England and Wales except London currently stands at £1,660 per year compared with the £255 given in 1962 when the system of grants was set up. As the chart shows, however, although the money value of the grant has increased 6.5 times over the past 20 years, its value relative to the retail price index has fallen by 20 per cent since 1962 and by 11 per cent in the four years since 1979. In addition, NUS points out that the retail price index is designed to measure the value of the average family income and therefore fails to account adequately for students' living expenses.

Melanie Kee

Israeli research

Neglecting the basics

Rehovot

ISRAELI basic research has dropped to "a dangerously low level" according to Professor Haim Harari, who heads the planning and grants committee of Israel's Council for Higher Education. Speaking at the recent Bat-Sheva de Rothschild seminar on science policy, Harari argued that in the past ten years Israel has gone from an over-emphasis on basic research to neglect.

This shift stems from the financial plight of Israeli universities, which now have to make do with a total annual budget of \$400 million, roughly what a single American university, Stanford (with its medical school and accelerator), spends annually. Some 70 per cent of the Israeli university budget comes from government, but how the government's share is distributed depends solely on members of the planning and grants committee, who, once appointed by the Minister of Education, are free to allocate funds as they see fit.

The government does decide, however, on the total size of its financial commitment, which has not kept pace with the growth of universities and with rising costs. As a result, local institutions of higher education must spend almost all their available funds on salaries and maintenance, leaving little for such "luxuries" as basic research.

There is a great deal of money from government ministries and booming science-based industries for applied research, but neither will support basic research, with the result, Harari says, that many professors who lean in that direction are "unemployed". To be sure, they still have their salaries, but they have no funds for equipment, technicians and the like.

Admittedly influenced by the fact that he is a theoretical physicist interested in elementary particle physics, what he terms "perhaps the most basic research", Harari is alarmed by the trend away from basic

research. So he and his colleagues on the planning and grants committee have decided to "confiscate" three per cent of the money allocated for higher education and to set it aside for basic research. They hope that this will rise to five per cent in the years ahead.

Professor Harari points out that Israel's booming computer-related companies are an outgrowth of computer research at the Weizmann Institute in the 1950s and 1960s, while Israel's aircraft industry could not have been developed if the faculty of aeronautical engineering had not been established at the Haifa Technion long before there was thought of such an industry.

But "today's basic research", Professor Harari warns, "does not provide the basis for economic advances in the year 2000". Participants in the seminar also heard a spirited defence of basic research from the Minister of Science and Development, Professor Yuval Ne'eman, who, like Professor Harari, is a physicist.

Although he actually has access to less money than Harari (the science ministry's total budget this year is only \$9 million), Professor Ne'eman intends to do all he can to ensure that some Israeli scientists are able to do basic research or, as he puts it, "to grope in the dark".

There were no complaints about the present situation from those at the seminar who are primarily connected with applied research and science-based industry, among them the Chief Scientist at the Ministry of Industry and Commerce, Professor Arie Lavie, and the head of the Elron electronics company, Mr Uzia Galil.

Professor Lavie reported that large-scale Israeli Government support for research projects linked to science-based industry had been very successful, with some 50 per cent of them ending with saleable products, more than \$1,000 million of which were exported last year. Moreover, he reported, bilateral applied research projects had long been supported by the US Government and new agreements meant that such support would also be forthcoming from Canada and France. But by far the most important source of research funds was provided by stock sales. In just one month, Professor Lavie said, three Israeli science-based industries had raised \$100 million on the New York Stock Exchange, and another three expect to raise \$50 million more in the immediate future.

According to Uzia Galil, the money is available for investment in good science-based projects but there are not enough good projects to go round. A lot of new ones, he predicted, will be initiated by expatriate Israelis who, having developed electronics and computer companies in the United States, are now anxious to do the same thing in the land of their birth.

Nechemia Meyers

Polish pollution

Changes may come at last

POLAND's Council of Ministers last month bent to the need for a government agency dealing with the environment. It adopted a draft resolution breaking up the Ministry of Administration, Local Economy and Environmental Protection and establishing a Main Office for Environmental Protection and Water Resource Management.

The need for a separate body has been apparent for some time, in spite of censorship during the 1970s on all environmental issues. By making the new body a main office and not a ministry, the Council of Ministers has tacitly accepted the urgency of the problem — as a main office, the new body will be answerable to the Prime Minister.

The proposed new body will face the three endemic problems of all Polish planners: lack of resources, lack of verified data and lack of public confidence. Until 1980, little was published generally about environmental pollution. Although partial statistics were available to experts and officials, the residents of the major industrial conurbations, especially Silesia and the Krakow area, found themselves having to cope with the qualitative aspects of the problem without objective data on the hazards. (It was widely believed, for example, that vegetables grown too near the Nowa Huta steel mills were a health risk because of dust from the scrap-metal recycling plant.) In some cases the underground press reported on major health hazards, in particular the sewage pollution of the Bay of Gdansk, but these "independent" commentators may often have underestimated the hazards.

During the comparatively open Solidarity period, the Krakow-based Polish Ecological Club was instrumental in January 1981 in having banned one of the main sources of pollution, hydrogen fluoride from the obsolete smelting shops at the Skawina aluminium works which had been emitting hydrogen fluoride in quantities sufficient to corrode gold art treasures in Krakow's Wawel Castle museum.

This openness has, to a certain extent, continued under martial law, gradually building up a picture of ecological near-disaster, with rivers, lakes, coastal waters, soil, forests and residential areas all at risk. But funds to rectify matters have been scarce — and, as the presidium of the Central Committee's Commission for Health and Environmental Protection noted last November, there remain "considerable discrepancies" between the official reports on remedial measures and the perceptions of the local population.

Indeed, as Politburo member Zofia Grzyb told that meeting, the shortage of resources would make it impossible to realize many recommended measures in the

Australian-USSR relations

Sanctions lifted

Canberra

As anticipated, the federal government last week lifted all sanctions against the Soviet Union originally imposed after the intervention in Afghanistan (see *Nature* 21 April, p.648). Cultural, sporting, scientific and academic exchanges can now be resumed. The Minister for Foreign Affairs, Mr Bill Hayden, said that the decision had been taken "in the wider interests of involving the Soviet Union in a more productive relationship". The move will no doubt make reparation for the expulsion in April of a first secretary at the Soviet Embassy, Mr Valeriy Ivanov, alleged to be a spy.

Vimala Sarma

near future. A plea last autumn by Deputy Premier Roman Malinowski for "close cooperation" with Czechoslovakia and the German Democratic Republic to save 60,000 threatened trees in the western province of Jelenia Gora, where pollution and insect pests have a synergetic effect, seems to have been a broad hint that Poland's neighbours might help bear some of the cost of setting right the damage to which their pollution contributes.



By February this year, the State Environmental Protection Inspectorate produced what was described in official circles as a "pessimistic" report concluding that, in many cases, irreversible environmental changes were already under way. Surface waters, especially rivers, seem to be in a particularly bad state, with only 1 per cent of the total length of Poland's rivers in the first category of cleanness (fit for human consumption) while the Vistula has second category water (fit for agriculture) only near its source. More than 60 per cent is below even the third category (little more than sewage). These figures were known to specialists in Poland as early as 1976, but have not been publicly released until now.

Now, there is growing public recognition of the health hazards of pollution black spots. In Plock province, there has been a call for "comprehensive investigations" into the high morbidity rate, particularly among children under two years of age. Last month a 10-man environmental subcommission of the Sejm (Parliament) visited Katowice, where it declared itself "shocked" by the workers' housing estates, with "windows only a few metres from sources of pollution".

Recently, moreover, a leading ecologist, Professor Henryk Zimny, has argued that pollution control is cost-effective, a factor the new environmental body will doubtless find valuable when negotiating funding. Some 600,000 million zloty, says Professor Zimny, are lost annually to the Polish economy for lack of pollution control. If only 10 per cent of this sum could be spent on protective measures in the immediate future, these losses could be halved.

Vera Rich

US nuclear power

NRC divided on emergency plans

Washington

THE beleaguered nuclear power industry narrowly averted a major political setback last week by persuading the Nuclear Regulatory Commission (NRC) to rescind a threat to close two power plants at Indian Point in New York. NRC had warned last month that the plants would be shut down because the Federal Emergency Management Authority (FEMA) considered emergency evacuation plans for the area to be inadequate (see *Nature* 12 May, p. 101).

Had NRC not changed its mind, the Indian Point reactors would have become the first operating plants to be closed down on the basis of deficient emergency plans. The commissioners decided by three votes to two to keep them open because a new report by FEMA said the plants' operators had stepped up planning efforts and promised to hold an emergency exercise within the next 60 days.

The close vote reflected sharp disagreements among the five commissioners. Mr James Asselstine, who voted to close the plants, said that nothing in the new report from FEMA indicated that a satisfactory emergency plan had yet been devised for Indian Point. In these circumstances, the decision to rescind the shutdown threat "made a mockery" of NRC's own rules.

Mr Victor Gilinsky, the other commissioner to vote for closure, complained that despite the uniquely high population in the vicinity of Indian Point, it was the only nuclear site that had consistently failed FEMA's tests of emergency preparedness.

But Mr John Ahearne, a commissioner who had been expected to recommend a shutdown but who changed his mind "reluctantly" after reading the new FEMA report, said enough progress had been made over recent weeks to suggest that a satisfactory emergency plan could be devised in the near future. A principal obstacle — the reluctance of a nearby municipality to participate in emergency planning — seemed to have been overcome by an undertaking by the State of New York to take over the county's emergency functions.

The commission's decision was being closely watched by the nuclear power industry and its opponents as a test of NRC's attitude towards the strict new evacuation regulations it introduced after the Three Mile Island accident in 1979. In a number of recent cases, notably at the Shoreham plant in Long Island, municipalities opposed to nuclear power have attempted to prevent reactors from operating by refusing to participate in emergency planning.

Another significant victory was scored by the industry earlier last week when the Supreme Court upheld the authority of NRC to disregard, in hearings on the licensing of nuclear plants, the possible impact

on the environment arising from the long-term disposal of nuclear waste. The Natural Resources Defense Council had challenged the commission's assumption that a method for disposing safely of long-term wastes would eventually be found.

Justice Sandra Day O'Connor, said the Supreme Court, in a unanimous decision, had found no reason to fault NRC's prediction that uncertainties surrounding the development of a permanent waste disposal depository were unlikely to affect the outcome of any individual licensing decision. In reaching this conclusion, she said, NRC was making decisions "within its area of special expertise, at the frontiers of science". In such circumstances, a court should be at its "most deferential".

At issue in the case was a table contained in the generic rules issued by NRC to aid licensing hearings for individual reactors. The table, S-3, consists of a numerical compilation of the estimated resources used and effluents released by fuel cycle activities supporting a year's operation of a typical light water reactor. In compiling the table, NRC decided to assume that the permanent storage of waste would have no significant environmental impact.

Peter David.

Kestrel under threat



THE Mauritain kestrel (above) is one of several bird species in Mauritius threatened with extinction: the surviving population is said to be numbered in single figures. Mr Robin Brown, of UK Central Television, is forming a committee to create a bird sanctuary covering the Black River Gorge in the south west of the island. The project has the support of the Mauritain Prime Minister and is backed by local businessmen and conservation groups. □

How to run elections

SIR — Professor May's article (*Nature* 5 May, p.16) on electoral procedures for ranking candidates A, B and C is of practical value in a country such as the United States or France which has to elect one person to be president. We must, however, beware of thinking that it has any relevance to the election of parliaments. For that purpose, *no* method of electing only one person at a time can ensure that the elected body reflects the wishes of the people electing it. Not even that a party ranked first by a substantial majority of the voters will have a majority of the elected members.

The most that Condorcet, Borda or any other system can do in a single-member constituency is to ensure that the member is elected with the consent of at least half the voters. Hence it would be theoretically possible for one party to win all 650 seats in the House of Commons if supported by only one more than half the voters in each constituency. But the same party could have much more support and yet lose the election — by winning a few seats with huge majorities while its opponents slipped into many seats by tiny ones.

To make the great majority of votes effective, and so to make the elected body reflect the opinions of the great majority of the voters, it is essential to elect several members together, for example all those for Bristol or for Bedfordshire as one constituency. Only then is it possible to give representation both to the majority and to any large minority, and to give a large majority more representation than a small one. The worthwhile discussion is on how best to do that.

ENID LAKEMAN

Tunbridge Wells, Kent, UK

SIR — Robert May's article on voting systems is timely: there is currently discussion in the University of Cambridge about the method of electing members of the Council of the Senate, occasioned by an incident last year when the election of four members had to be disallowed because of an error in a ballot paper. We use the single transferable vote (STV) system specified by Parliament in 1918¹ with the modification, introduced in 1952, that it is also to be used even if there is only a single vacancy.

The ensuing discussion has revealed that many, perhaps most, electors do not understand either the principles of the system or its several virtues. Thus it does not pass the first test of an electoral system, that it has the confidence of the voters.

As May points out, there is no way round the Condorcet (or "voting") paradox when electors place candidates in order of preference. Let us therefore stand the problem on its head and ask not "how can we design a voting system to minimize the occurrence of the paradox?" but "how can we design a voting system which will maximize the information each elector may contribute subject to the restriction that the

voting paradox must not appear?"

Theorem: The voting paradox can arise if and only if voters are allowed to place in order of preference three or more candidates.

Corollary: If the voting paradox is to be impossible, the most information that any voter can be allowed to communicate is "I prefer each of A,B,C, . . . to each of P,Q,R, . . .", where A,B,C, . . ., P,Q,R are the candidates.

The resulting voting system is simply that in which if there are n candidates for k places each voter votes for any subset of the candidates he pleases, and the candidates' total votes are then counted in the ordinary way. Each voter can vary his strategy from voting only for A if the election of A is his sole objective, to, at the other extreme, voting for everyone except A if the non-election of A is his sole objective.

A common version of this system is to limit each voter to a maximum of k candidates for whom he may vote; an extreme version is to limit each voter to naming one candidate only, which has a "proportional representation" effect. Somewhere between allowing only one name and allowing any number there should be a simple system which would command wide acceptability in an academic community.

It is therefore interesting to note that by Act of Parliament² in 1854 the University of Oxford was required to elect the members of the Hebdomadal Council (the equivalent of Cambridge's Council of the Senate) by just such a limited-vote system, the limits being prescribed as "for One Vacancy, One Vote [that is, one name]; for Two or Three Vacancies, Two Votes, for Four Vacancies, Three Votes; for Five or Six Vacancies, Four Votes; Provided always, that no Elector shall give more than One Vote for any One Candidate."

However, in 1971 the Hebdomadal Council, calling this system "unnecessarily complicated", proposed one vote for each vacancy in future. The university concurred without a single dissentient voice. It seems indeed that what most people require of a voting system is that they can understand it.

Under the STV system in which voters can select any, or all, of the n candidates and place them in order of preference, the total number of ways of completing a ballot paper is given by x_n where

$$x_n = n(x_{n-1} + 1), x_1 = 1$$

For $n = 10$ this is 9,864,100, and gives an indication of how the STV system provides the voter with too many options. The solution of this recurrence relation involves the partial sum of the common series for e ; indeed, $x_n = \text{integral part of } (e \cdot n! - 1)$ is, remarkably enough, the exact solution for all values of n , even $n = 1$.

A.W.F. EDWARDS

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1. *University Elections (Single Transferable Vote) Regulations* Statutory Rules and Orders No. 1348 (1918).
2. *Victoriae*, 17, 18 c. 81, XXI.

The wine dark sea

SIR — Homer's references to a "wine-coloured sea" have been a puzzle with such varied "solutions" as the absence of a word for "blue" in their language (manifestly incorrect), through congenital colour-blindness in the Achaeans (patently unlikely) to an outbreak of red-coloured marine algae (possible, but not long-lasting). It is curious that a much more earthy explanation has been overlooked.

The original meaning of the word *symposium* (συμπόσιον) was a convivial gathering for the exchange of interesting conversation. It is known that the wine was not usually taken pure but only after being diluted with as much as six or even eight parts of water. The geology of the Peloponnese includes large formations of marble and limestone, so that the ground water must have been alkaline, and where it was sufficiently alkaline it would have raised the pH enough to change the colour of the wine from red to blue. What this did to the taste is irrelevant inasmuch as everything depends upon what one is used to.

We offer this suggestion to underline the value of communication within the various realms of academia.

R.H. WRIGHT

R.E.D. CATTLEY

Vancouver, Canada

Lightning strike

SIR — The lightning-related phenomenon reported by Burbidge and Robertson (*Nature* 300, 623-624; 1982) is the only instance I have found, after an extensive search of the literature, which parallels descriptions of amorphous glowing complexes, about the size they describe, seen moving erratically over the surface of hills on the south face of the Himalayas. Paljor Ngodop (Anchorage, Alaska) grew up in the kingdom of Mustang, and in childhood observed such glowing shapes several times. Children used to chase them and throw rocks at them; their elders warned against it, fearing that the objects would turn upon their pursuers.

I never reached Mustang, but in 18 months in the Solu Khumbu and other border points in northern Nepal, I was struck by the daily regularity and long continuation of lightning, at least in spring and summer, on the south side of the highest Himalayan ridge. Lightning-related effects must be correspondingly more common: these are common enough to have been incorporated into the region's folklore, and are categorized as *shipa* — apparent but nonmetabolic organisms — a grouping also including the yeti and dragons (perhaps colloquialisms for sightings of the rare Himalayan griffon). Tibetan scholars should be alerted to any description or reference in Tibetan literature.

W.S. HOME

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Eclipse of the free quark charge

The existence of electric charges which are fractions of the elementary electronic charge has been widely canvassed, but has now been cast in doubt. The search has however been a spur to ingenious experiment.

SINCE the demonstration by Robert Millikan that the electric charge acquired by a droplet suspended against the force of gravity by an electrostatic field is invariably a multiple of some constant amount, the same technique has been variously held up as a demonstration of the precision of physical measurements and as proof of how important natural constants can be determined directly. Only in recent years has the complaint that Millikan may have improved on nature in presenting his results been considered seriously, and then only in the spirit that there have accumulated so many other pieces of evidence bearing on the constancy of the unit charge, that the elegance of Millikan's experiment remains a model for the rest of us.

This was true at least until the hunt began for the particles called quarks which, when their existence was first predicted by Murray Gell-Mann, were known to have only one distinctive property — a fractional electric charge, either one-third that of an electron or twice as much, plus or minus. That, it will be recalled, was the period when there was such uncertainty about the masses of the quarks that schemes for hunting them with vacuum cleaners beneath electric fences, or dredging for them on the sea-bottom, were all the rage.

William Fairbank from Stanford University had a better idea. He set out to repeat Millikan's measurement but with extra ingenuity and with much greater precision. Fairbank's announcement, nearly a decade ago, that small spheres of niobium suspended by magnetic levitation appear to behave in electric fields as if they carried fractional electric charges seemed, at the time, as neat a pointer to the existence of quarks as there could be. Only as the lesson has sunk home that unbound quarks do not exist has his elegant experiment been counted as a challenge. For if Fairbank's apparently fractional charges cannot be free quarks, what are they?

The consequence has been a prolonged and detailed examination of the side-effects that may vitiate the interpretation of Fairbank's measurement. All kinds of sources of systematic error have been examined. A vivid measure of the exhaustive way in which the issue has been tackled is that one of the most serious sources of error may be the variation from place to place of the electrical properties of the surface of a metallic object. Specifically, there may be patches on a metallic surface whose prop-

erties differ from the remainder, perhaps because the crystal structure in the locality enables electrons to be detached with a voltage a little smaller or larger than the average work function.

Variations of the work function of a few tens of millivolts are well documented. Their consequences for measurements in the Fairbank mould are obviously important. If, for example, it is planned to drive a suspended charged object in one direction or another by means of a uniform electric field generated by applying some voltage to a pair of parallel metallic plates, patches of abnormal work function will undermine the assumption that each surface is an equipotential surface and thus that the electric field between them is uniform. So how can unavoidable difficulties such as these be avoided?

This is the challenge to which have risen D. Liebowitz, M. Binder and K.O.H. Ziock from the University of Virginia at Charlottesville. In *Physical Review Letters* on 23 May (50, 1640; 1983), they describe an experiment which is in many ways simpler than others in this genre but in which variations of the surface properties of suspended spherical objects can be ironed out. This is not the first attempt to allow for departures from electrical spherical symmetry, whose consequences can spuriously endow the sphere with the appearance of extra electric charge. Attempts to swamp this effect, both at Stanford and at Charlottesville, by making suspended spheres spin rapidly have apparently failed, chiefly because of the precession thus induced in the magnetic field.

The Charlottesville group has worked with steel spheres 0.2 mm in diameter, gigantic compared with Fairbank's particles of niobium dust. To avoid the complications arising from precession, the group has devised an apparatus in which the spheres are suspended by a vertical magnetic field and driven vertically up or down by means of the electric field between two horizontal condenser plates. The plan is to make the steel spheres spin also about a vertical axis, so that precession generated by patches on the surface of the spheres should be negligible.

Naturally, the whole equipment is evacuated. The electric field between the condenser plates (only 2 cm apart) is made to oscillate twice a second, and an arrangement made for a supplementary magnetic field provided by a coil to prevent the physical oscillation of the suspended ball

by means of a servo-system driven by an optical sensor. The variable current in the coil is thus a means of inferring the electrostatic force on the suspended ball and thus, if patches are irrelevant, of the net electric charge it carries. To ensure that patches are irrelevant, the steel spheres are made to spin rapidly by means of an oscillating magnetic field.

The experimental arrangement is simple as well as elegant, but its designers plaintively note the difficulty they have encountered in telling not merely how quickly their steel spheres are spinning but, by simply looking, whether they are spinning at all. This is why they scratched the surface of one of their 24 steel spheres to infer that after a few minutes, their spheres were spinning at 600,000 revolutions a minute.

The results would serve only to make Millikan even more satisfied with himself than he is said to have been in real life. It is possible to induce extra electric charge on the steel spheres, spinning or otherwise, by means of ultraviolet light. The authors of the experiment report that their spinning spheres acquire only integral electric charges, and then only after these have been deliberately induced. Specifically, they "never observed charge changes that were not an integer multiple" of some highest common factor, roughly the elementary charge. The supposed elementary charges of spheres not spinning vary about the known value of the elementary charge, in some cases by as much as a third of the electronic charge, but the same spheres spinning give elementary charges much more closely bunched about the expected value. Something like the patch effect must be at work.

Where does this leave Fairbank's data? The authors of this new measurement generously say that steel is not niobium, so that what they have done does not undermine the earlier claim that fractional charges exist. It is possible to argue that the conditions of the two experiments are so different that fractional charges were somehow excluded from the Charlottesville measurement. Whatever the truth, the onus now lies with Fairbank to eliminate the patch effect with niobium spheres. If the result should be that fractional charges seem not to exist, Fairbank will at least have had the fun of hunting quarks for more than the past decade, and the knowledge that he has stimulated a series of ingenious experiments that otherwise would never have been done.

John Maddox

Immunology

The T-cell receptor — an important step forwards

from Alan Munro

It now seems likely that the biochemical nature of the T-cell receptor will soon be solved. The reason for this optimism is that three groups have monoclonal antisera which identify biochemically related molecules on T cells and there is persuasive evidence that these molecules represent the part of the T-cell receptor that is involved in specific recognition¹⁻⁴.

The strategy adopted rests on the following argument. Different clones of T cells recognize and respond to different antigens. Therefore there must be on the surface of a T cell, molecules which determine the specificity of this interaction and which must be unique to particular clones of cells. In theory it should be possible to immunize with cells from a T-cell clone and to use monoclonal antibody technology to obtain antibodies that react with the unique features of the T-cell recognition structures. This approach avoids making assumptions about the biochemical nature of the T-cell receptor, for example, whether it contains immunoglobulin V_H regions or not⁵.

In practise it has been possible to obtain monoclonal antibodies that react with T cells of the immunizing clone but with no other T cells, or any other cells for that matter. Such clonotypic antisera need not necessarily be reacting with the T-cell receptor, however, particularly as the clones of T cells used in the experiments are derived from T-cell hybridomas or long-established T-cell lines and may have accumulated somatic mutants in surface molecules unrelated to the T-cell receptor. It is encouraging to show that the clonotypic antisera inhibit the response of the T cells to their specific antigen, though not sufficient, as inhibition of response might arise as a secondary effect following the binding of antibody to an irrelevant surface component of the cell.

One group has taken a more direct approach to this problem and have presented two additional pieces of evidence which strongly support the concept that their clonotypic antibodies are indeed reacting with the T-cell receptor³. The first is that the clonotypic antibody will block the binding of T-cell hybridoma cells to the relevant antigen presented on antigen-presenting cells, that is the antibody blocks primary recognition of antigen. The second is that among subclones of the original immunizing clone, some of which no longer respond to the specific antigen, there is complete concordance between a positive reaction with the clonotypic antisera and the ability to bind to, and respond to the antigen. All the evidence taken together points strongly to reaction of the

clonotypic antisera with the part of the T-cell receptor that determines specificity of T-cell interaction with antigen.

The other striking feature of this work is that the clonotypic antisera isolated in different laboratories all react with a disulphide-bridged dimer of 80–90,000 molecular weight which in most cases has been shown to be a heterodimer of chains in the 40–50,000 molecular-weight range. The molecule has been found in mouse and human T-cell clones and in T cells with helper and cytotoxic activity. Even though there appears to be only 20,000 copies of the clonotypic molecule per cell, with monoclonal antisera to aid in the purification and T-cell tumours or hybridomas to provide endless supplies of material, it will not be long before information on the molecular biology of these molecules becomes available. We will then at last know which genes code for the variable

part of the T-cell antigen receptor and ultimately the mechanisms of generating the T-cell repertoire.

Finally, the clonotypic antisera have been used to confirm the intimate association of the T3 molecule of human T cells with the antigen receptor. T3 is a glycoprotein of molecular weight about 20,000 which is first expressed on cells in the thymus late in T-cell development and remains expressed on all peripheral T cells. Antibodies to T3 inhibit T-cell function and can act as T-cell mitogens (see ref. 2 for references). It has now been shown that modulation of T3 with monoclonal anti-T3 antibody co-modulates the clonotypic molecule and vice versa². So far no other T-cell surface molecules have been identified that co-modulate with T3 or the clonotypic molecule. The implication is that T3 and the clonotypic molecule form a noncovalently linked complex on the surface of the cell but the biological significance of this is at present unclear. □

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Neurophysiology

Anatomical logic of retinal nerve cells

from Nicholas V. Swindale

It is often assumed that neurones simply add their inputs, so that above a certain threshold, firing rate is proportional to the difference between the number of excitatory and inhibitory inputs active. Although the occurrence of this sort of linear summation can be inferred^{1,2} there has been very little direct study of the issue. Empirical investigation is difficult, since it is rarely possible to record from even one, let alone several or all of the inputs to a cell which is also being recorded from. An alternative approach, pioneered by Wilfrid Rall³, is to try to determine by theoretical means the kinds of operation that nerve cells might be expected to perform. If the dendritic membrane is assumed to be electrically inexcitable, as is probably the case with many mammalian neurones, then the problem can be solved given accurate information about the properties of the dendrites: for example their membrane resistance and capacitance, their shape, and the location and nature of the conductance changes induced by the synaptic inputs.

The theories laid down by Rall deal in the main with dendrites whose branches satisfy the equivalent cylinder condition, a mathematical simplification of cell shape which seems not to be valid^{4,5}. Recently

however, Koch, Poggio and Torre have applied Rall's analysis to some morphologically realistic models of cells. They devote two papers^{5,6} to an analysis of the properties of the different types of ganglion cell in the cat retina, using examples of cells taken from the Golgi-Cox-stained material of Boycott and Wässle⁷, while two further papers^{8,9} form a timely contribution to the recent debate^{10,11} about the possible function of dendritic spines.

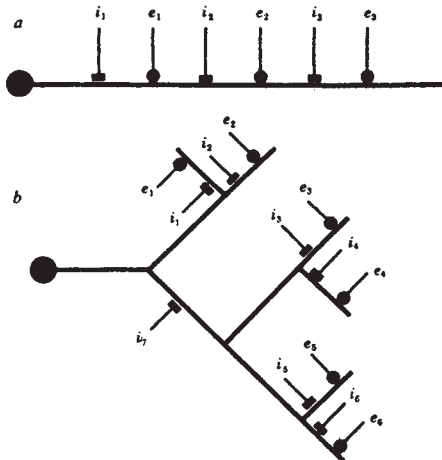
Using currently accepted estimates of dendritic membrane resistance and capacitance for mammalian nerve cells (2,500 ohms cm² and 2 μF cm⁻² respectively), and assuming conductance changes induced by presynaptic activity to be in the range 10⁻⁶–10⁻⁹S, Koch *et al.* discuss the conditions in which cells may be expected to integrate linearly or nonlinearly.

Estimation of a single electrotonic space constant for a particular dendritic tree turns out not to be a good way to describe its properties, since the branching pattern of a cell can give rise to substantial asymmetries in the pattern of current flow from one part of the dendritic tree to another. Instead, Koch *et al.* calculate transfer resistances (which are to be thought of as shorting out the signal) for different pairs of points on the dendritic tree, and then use

these values to decide how a cell might function. A cell that is sufficiently small will be electrically homogeneous and the details of its shape will be of little importance in determining how it will function. Larger cells however have dendritic trees that can be partitioned into more or less independently functioning regions that Koch *et al.* term subunits. These are so defined that transfer resistances between points within a subunit are high, implying isopotentiality and good electrical communication; while transfer resistances between points in different subunits are low, indicating relatively poor electrical communication. A consequence of this is that the cell is more likely to behave in a logical nonlinear way and less like a simple adding device. Within a subunit for example, there will be rapid saturation of the response to many excitatory inputs, approximating to a logical OR function, while inhibitory inputs to a subunit will be effective mainly locally, performing an operation akin to a logical AND.NOT function (the subunit gives an output if there is an excitatory input active and the inhibitory input is not active). The interaction between different subunits is linear. (See the figure.)

In the cat retina, alpha cells and peripherally located beta cells have many (≤ 30) large subunits, while beta cells within a few millimetres of the *area centralis* have only a few small subunits. It is obviously tempting to suppose that the subunit structure of the alpha cells could be one cause of the nonlinearities in the physiological behaviour of Y cells¹ (which are probably identical with the alpha cell class¹²), whereas the near absence of subunits from centrally located beta cells would be consistent with the linear integration of light stimuli by X cells (probably identical with the beta cell class). It is less easy to rationalize the fact that peripherally located X cells are no less linear in their physiological properties than central ones¹, though since subunit behaviour disappears with increasing membrane resistance it could be that this is simply higher in X cells than in Y cells, or that it increases with retinal eccentricity.

Dendritic spines, on the basis of this kind of analysis, turn out to behave essentially as single subunits with a very high input impedance. This leads to some interesting new speculations about their possible function. Because of their small volume, even a small conductance change (for example, one equivalent to the action of one or two acetylcholine quanta at the neuromuscular junction) will cause the voltage in the spine head to saturate at the equilibrium potential for the new conductance. This means that the current injected by the spine neck into the dendrite will be relatively insensitive to the amount of transmitter released by the presynaptic axon. This 'digitization' of synaptic inputs could make nerve cells less sensitive to quantal fluctuations in the amount of transmitter released, and thus more reliable. Another possibility is a very



Performance of complex logical operations by dendrites receiving excitatory inputs (●) and inhibitory inputs of the shunting type (○). (Taken from ref. 5.) Any inhibitory input vetoes only more distal excitation and does not affect other inputs closer to the cell body. If the inhibitory interaction is described as an AND.NOT gate, the operation performed in *a* could be read as

$[e_3 \text{ AND NOT } (i_1 \text{ OR } i_2 \text{ OR } i_3)]$
 OR $[e_2 \text{ AND NOT } (i_1 \text{ OR } i_2) \text{ OR } (e_1 \text{ AND NOT } i_1)]$
 The operation implemented in *b* would be
 $(e_1 \text{ AND NOT } i_1) \text{ OR } (e_2 \text{ AND NOT } i_2) \text{ OR } [(e_3 \text{ AND NOT } i_3) \text{ OR } (e_4 \text{ AND NOT } i_4) \text{ OR } (e_5 \text{ AND NOT } i_5) \text{ OR } (e_6 \text{ AND NOT } i_6)] \text{ AND NOT } i_7$

specific veto of the excitatory input to a spine by an inhibitory input located at the base of the spine (an arrangement estimated to be present on about 5–20 per cent of spines). This veto can be highly specific in time, as to be effective the inhibitory input has to occur within about 120 μ s of the peak of the excitatory conductance change.

Further speculations concern the behaviour of inhibitory inputs. In general these obey the 'on-path' condition established by Rall¹³, and which says that an inhibitory input can have an effect that is specific for excitatory inputs more distal on the same branch of the cell. Koch *et al.* go on from this to uncover some interesting differences in the behaviour of inhibition where the conductance change has a reversal potential either close to the resting membrane potential of the cell (so-called 'shunting inhibition'), or below it. In the latter case the optimum location for inhibition is always on the cell body, and inhibition will tend to subtract linearly from excitation. Many inhibitory nerve cells seem to obey this rule: basket cells in the cerebellum and cerebral cortex for example make inhibitory connections exclusively with cell bodies, suggesting that they are able to minimize their energy requirements for transmitter synthesis as a result. If, on the other hand, inhibition is of the shunting type, the interaction between excitation and inhibition becomes nonlinear and the optimum location moves towards, and becomes specific for excitatory inputs on branches distal to the inhibition.

That this type of inhibitory veto, equivalent to an AND.NOT operation, could be performed by an excitatory and an inhibitory input close to one another was first pointed out by Torre and Poggio¹⁴.

Koch *et al.* have shown however that this veto can be extended to all inputs more distal than the inhibitory one, allowing different parts of the dendritic tree, even in cells that lack subunits, to function independently. The AND.NOT operation is of particular interest as there is experimental evidence that it is carried out by retinal cells that are direction selective¹⁵. The cell that seems morphologically best suited to do this in the cat retina is the delta cell, a prediction that should be testable by intracellular recording and dye injection.

The picture of the nerve cell as a device that can perform a variety of logical operations on its inputs, rather than just a single thresholding operation, is simple and attractive, but how likely is it to be correct? A problem that besets all such theorizing is the scarcity of relevant biophysical data: for example the estimate of membrane resistance of 2,500 ohms cm^2 is based on studies of cat spinal motoneurons, and no one has yet estimated it for cat ganglion cells. A value greater than 8,000 ohms cm^2 , though less probable, is not implausible, and would abolish subunits in most cells, although the nonlinearities resulting from 'on-path' inhibition would remain. The assumption of electrical inexcitability is still untested, while the re-invasion of the dendritic tree by the axonal spike may also cause complications. Looking at the problem from a different angle, one can see that while the computing power of a single nerve cell might be increased by perhaps an order of magnitude, significant additional demands would be made on developmental mechanisms which guide the positioning of synapses on dendrites, and on the reliability of the signals feeding into particular parts of the dendritic tree. Nevertheless, it seems likely that there will be many cases in which these demands can be met, and that nerve cells will make use of the increased computing power available to them. The possibility that the shape and branching pattern of a cell's dendrites may imply more than just a certain density and spatial extent of connections is too attractive to dismiss. □

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Seed viability

Seeds of thought for plant conservationists

from Peter D. Moore

A RECENT paper¹ describing the successful germination during greenhouse tests of the fen violet, *Viola persicifolia*, in soil samples taken from Wicken Fen, Cambridge, where the violet had been believed to have been extinct since 1916, raises two important questions. First, for how long can seeds remain viable? And second, how should conservationists define extinction for a plant species at a site?

Some answers to the first question come from discoveries of plant seeds in datable archaeological contexts. The *Canna* necklace, found during the excavation of a tomb in Argentina, comprised a string of rattles made of walnut shells (*Juglans australis*) containing *Canna* seeds². When kept moist and dark the *Canna* seeds germinated. Bone material from the same site was radiocarbon-dated at 530 years old. Godwin³, however, questioned the association between the bone (of a cameloid animal) and the necklace, and hence cast doubt upon the validity of this claim for an ancient viable seed. Godwin's doubts stimulated Lerman and Cigliano⁴ to redate the material, this time using the *Juglans* nutshell. They obtained a date of 620 ± 60 years ago, thus confirming the antiquity of the viable seed.

Only too often, however, the association between artefact and seed is rather tenuous and hence the dating of the seed unreliable. Such is the case with the 3,000-year-old canoe found near Tokyo with 'associated' viable water lily seeds. These could easily have been derived from later contaminant sediments⁵.

Experimental work on seed viability must, by its very nature, be long term and few have had the patience or foresight to set up seed-viability experiments. There was the Kentish schoolboy, Peter Ashenden, who entombed a bottle of wheat grains in a new wall built around his school in 1872. This time-capsule eventually came to light a century later, during demolition works, but when the seeds were tested at the ARC Unit in Developmental Botany, Cambridge, they were found to be non-viable⁶.

Just eight years after Peter Ashenden had begun his experiment in England, Professor W.J. Beal began a more ambitious and well documented experiment in Michigan, USA. Twenty samples of selected seeds were mixed with moist sand, placed in uncorked bottles and buried, neck downwards (to avoid waterlogging), in a field. For the first 35 years one sample was removed and tested every 5 years, but subsequently the interval has been increased to 10 years. The latest sample to be removed (the fourteenth bottle) was recovered in

1980, a century after burial, and a total of 27 of the original 1,000 seeds (mainly *Verbascum blattaria*) germinated⁷. The experiment could be criticized, however, on the grounds that open-necked bottles could permit access by earthworms and other soil arthropods which carry seeds in their gut⁸.

The sealing of seeds in walls has been going on, albeit inadvertently, for some considerable time in areas far removed from Kent. In California and Northern Mexico, for example, the adobe bricks of buildings contain the seeds from the mud of which they were built. In a recent study, Spira and Wagner⁹ examined extracted seeds from the bricks of 20 adobe buildings constructed between 1769 and 1837. Most of the seed samples had been recovered by archaeologists working 50 years ago and had been stored dry since then in glass vials at room temperature. Of about 40 species tested, 7 were found to have at least one viable seed (tested chemically) and 1 species, *Medicago polymorpha*, was successfully germinated to produce a seedling. It is open to question whether the viability would have been greater if samples had been taken directly from the adobe bricks instead of from storage tubes.

The possibility of long-term survival of seeds buried in soils or compacted in mud

brings us to the question of defining extinction of a plant species at a site. The loss of the vegetative stage in a plant's life history, even if its absence continues for several decades, does not necessarily prove that the species does not survive as dormant seed.

The importance of this possibility was realized when a plant species supposedly extinct in Britain, the fen ragwort (*Senecio paludosus*), was re-discovered in East Anglia in 1972 after an apparent absence of about 70 years¹⁰. Survival of buried seeds seemed a more likely cause of its re-appearance than re-invasion from elsewhere. In the more recent case of *Viola persicifolia*, almost certainly the seed had survived dormant in the soil since the early part of this century. In such a situation the next question which must be faced is how one can best manage the site in order to provide suitable conditions for the dormant species to emerge from its latent state.

Perhaps the time is approaching when nature reserves will be established not simply on the basis of their current vegetation, but as a result of the potential interest of their soil seed banks. □

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Meteorites

Calcium- and aluminium-rich inclusions

from Derek W.G. Sears

IN recent months there have been new developments in our understanding of calcium-aluminium-rich inclusions (CAI) and the meteorites in which they are found. Meeker, Wasserburg and Armstrong¹ argue that some CAI have suffered considerable alteration by processes which occur on small parent bodies. This adds a new episode to the history of the objects and their parent meteorites. And in this issue of *Nature* (p.588), Bischoff and Keil² discuss several CAI they have found in the largest meteorite class, the ordinary chondrites. Although there are many earlier studies of occasional CAI (see, for example, ref.3), it is the fact that Bischoff and Keil found CAI in every one of the 11 ordinary chondrites they examined which is significant.

CAI are light-coloured inclusions varying in size from millimetres to centimetres^{4,5}, and which were first found in quantity in the Allende meteorite. The in-

clusions were found to have had extremely low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, indicating that they were one of the earliest substances to form in the Solar System⁶. They were also found to be rich in all the refractory elements⁷ and to consist of a number of calcium- and aluminium-rich minerals which had previously been considered either absent or rare in meteorites. In 1973 they were found to have anomalous oxygen isotope abundances⁸ and soon a whole plethora of isotopic anomalies were reported; elements such as Ca, Ti, Sr, Ba, Sm and Nd were all found to have relative isotopic abundances that were not explicable in terms of well-defined chemical processes or nuclear processes such as radioactive decay or cosmic-ray-induced nuclear reactions⁹. Moreover, excess ^{26}Mg and ^{129}Xe , the products of radioactive decay of ^{26}Al ($t_{1/2} = 0.75$ Myr) and ^{129}I ($t_{1/2} = 18$ Myr) respectively, were

found^{10,11}. The presence, at some time, of ²⁶Al in the inclusions is considered particularly significant as it may provide the long-sought heat source in the early Solar System.

What Meeker and co-workers¹ recently observed is that in those inclusions which are fairly coarse-grained and consist predominantly of the minerals pyroxene, melilite, spinel and plagioclase, much of the melilite appears to have grown out of the pyroxene. They interpret this as evidence for a solid-state reaction at elevated temperatures and pressures (metamorphism), since crystallization from a liquid or direct condensation from the nebula would produce the opposite relationship, that is melilite from which pyroxene would grow.

They observe a whole spectrum in the extent of metamorphic alteration, ranging from little or none, where the textures suggest that virtually all the inclusions crystallized from a melt, to two instances where most of the inclusion appears to be alteration product. Two inclusions are also described where the core is crystallization product and alteration has occurred from the outside inwards and consumed roughly half the inclusion. Other properties of the inclusions also support the concept of metamorphism, such as reaction textures between melilite and spinel, lobate-sutured grain boundaries and 120° interceptions between grain boundaries.

Meeker *et al.* face certain mass-balance problems — they need to invoke *ad hoc* means to introduce Ca and remove Ti — but their idea provides an attractive means of explaining some oxygen isotope data. Clayton *et al.*¹² found that different minerals from a single inclusion had different oxygen isotope ratios, suggesting that different minerals had received different amounts of exotic oxygen. Metamorphism would be expected to produce such a distribution because different minerals have different equilibration temperatures and reaction rates for oxygen exchange. The fact that different inclusions from the same meteorite have been metamorphosed to differing degrees indicates a rather complex sequence of events: metamorphism on a parent body, disaggregation and finally re-assembly,

presumably on a new parent body. Thus some CAI are as much the product of planetary processing as anything else, and the Allende meteorite has a considerably more complicated early history than most workers previously supposed.

Although it is probably the best known and studied meteorite, the Allende meteorite is actually a member of a very small meteorite class, the CV chondrites. In this issue of *Nature*², Bischoff and Keil show that there are many similarities between ordinary-chondrite CAI and Allende CAI. They have the same mineralogy and bulk compositions, and both meteorite classes have high levels of Na and K in CAI. Na and K are fairly volatile elements, whose presence is difficult to reconcile with high-temperature condensation or evaporation.

A major implication Bischoff and Keil

see in their observation is that it again brings out the similarities between diverse meteorite classes. It is certainly true that the majority of meteorites have many similarities: the composition of all the classes is basically solar — except for the most volatile elements, the compositional differences on which the classification of meteorites is based are in a quantitative sense very minor; they consist of essentially the same components; and they are all extremely old. But it is also true, as many authors have pointed out^{13,14}, that there are important compositional and isotopic differences between the classes, and in stressing the similarities it is important not to lose sight of the differences. □

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Biological materials

The hammer of the shrimp

from Paul Calvert

ONE of the things that keeps the weapons industries fat and happy is that each advance in armour requires a better weapon and vice versa. As a natural example of this process, a recent paper in *Journal of Materials Science* (17, 1939; 1982) by Currey, Nash and Bonfield describes the structure of the smashing limb of the mantid shrimps and of the armour plate that protects them from being smashed in return by their fellows.

The smashing limb is essentially a spring-loaded hammer with which an 80-mm shrimp can deliver a blow sufficient to break the wall of a glass aquarium, yet which remains smooth over many months of use because of the subtle way in which it is constructed.

To make a good hammer one needs a material which is hard (which we can think of as high in modulus and strong in compression) so that it does not flatten during the blow. At the same time it must be strong in tension to resist chipping and cracking during impact. The difficulty is that most minerals, the naturally available hard materials, are very weak in tension. It was for this reason that medieval cathedrals were built so that all parts of the structure were always in compression: if a column went into tension the cathedral fell down. The crustaceans solve this problem by embedding the mineral in an organic matrix of chitin fibres and protein, so compromising between hardness and rigidity of the mineral and the tensile strength and toughness of the matrix.

The shrimp hammer is interesting for the way in which the relationship between the two phases changes through the structure. The outer part of the face of the hammer is a layer of about 15 µm of hard heavily mineralized material backed by softer

layers of fibrous chitin. Although Currey *et al.* did not actually measure mineral content, the structure is quite reminiscent of teeth where the enamel is 95 wt per cent mineral and the underlying dentine is softer and less brittle with 70 wt per cent mineral. The phosphorus/calcium ratio in the hammer also decreases progressively towards the interior from a maximum of 0.36 at the surface to 0.03 deep inside, suggesting that hard calcium phosphates (probably hydroxyapatite) predominate at the surface while the softer but metabolically cheaper carbonate is used inside.

This principle of surface hardening is widely used to make steels hard and wear-resistant without also making them brittle, and to get abrasion-resistant coatings onto plastics. The soft underlying material prevents the tensile cracking that would destroy the hard material if it were used for the whole piece.

Having made its weapon for smashing snails, crabs and other shrimps, the mantid shrimp must also make its armour to protect it from the weapons of its brothers. The telson is a thick plate at the rear of the shrimp which takes blows during dominance fights. This has a much thinner mineralized surface layer so that the surface can flex and absorb the impact. What nature has apparently not managed is a lightweight sheet armour which can withstand the hammer, otherwise the mantid shrimp would be out of business. The lesson seems to be that attack is easier than defence. □

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Astrophysics

Gravitational lenses and the missing mass

from J. A. Peacock

THE four years since the discovery of the double quasar have seen a great burst of activity in research on gravitational lenses. Observers worldwide have been surveying the sky for more cases of multiple quasar images caused by intervening galaxies; meanwhile, the theoreticians have been busy calculating the expected number of these events and investigating the consequences of their previous neglect on our understanding of quasars.

The worry has been that gravitational lenses can cause large flux amplifications (a factor of about 10 in total for the double quasar); distant quasars are more likely to be affected, as they have more galaxies on the line of sight, so that spurious trends of quasar properties with redshift could be produced. Such work has concentrated on the effects of visible lenses — galaxies and clusters — but there is strong evidence that most of the mass in the Universe is dark: the so-called 'missing mass' whose presence is inferred from studies of the dynamics of galaxy clusters. Candidates for the missing mass include compact objects: burned-out remnants of an early generation of stars, or even primordial black holes, all of which can act as gravitational lenses. This possibility has been considered in a recent paper (*Astrophys. J.* **263**, 508; 1982) by Claude Canizares. He uses the observed properties of quasars to constrain the composition of the missing mass, and is able to show that the Universe cannot be closed by objects with masses greater than about 0.01 times that of the Sun ($M_{\odot}$).

Canizares' analysis makes use of some basic facts about the structure of the emitting regions in quasars. Crudely speaking, observed variability time scales show that the non-thermal continuum originates in a region $\sim 10^{-3}$ parsec in size, whereas the characteristic emission lines in quasar spectra come from a region at least 1,000 times larger. These numbers are highly uncertain and probably vary between different quasars; nevertheless, there is general agreement on the gross disparity in scale between these two emitting regions.

The difference is crucial because the effects of a gravitational lens will depend on the size of the object being viewed: small objects can be highly magnified, whereas extended objects with large angular sizes will be little affected. The critical angular size is approximately the angle through which a ray of light is bent by a lens which lies along the line of sight. This scales as the square root of the mass of the lens, so that lenses with masses in the range $10^{-2} M_{\odot} \lesssim$

$M \lesssim 10^5 M_{\odot}$ can strongly amplify the continuum source in a quasar, while leaving the emission lines unaffected. If such lenses exist, then distant quasars will be subject to a scatter in their line-to-continuum properties: either the quasar is not affected, or, if it is, only the continuum flux increases. Canizares can thus predict a minimum scatter in line-to-continuum properties, since an intrinsic scatter will only make the total scatter worse.

The effects on the continuum source alone are also detectable: a gravitational lens will cause a characteristic variability pattern consisting of an abrupt brightening of about 1 magnitude, which persists for typically tens of years, until the random velocity of the lens takes it out of close alignment with the quasar.

We are forced to adopt indirect arguments such as these to look for the signatures of undetected gravitational lenses because the relevant masses are so

low. Even a lens of $10^5 M_{\odot}$ causes an image-splitting of only one-thousandth of an arc second, which is on the borderline of what can be detected by very-long-baseline radio interferometry (VLBI). For more massive objects, VLBI can rule out the existence of a significant density of lenses; Canizares' contribution is to enable us to probe masses a million times smaller. His calculations require selection effects in observed samples to be quantified carefully for comparison with predictions, which complicate the conclusions. Nevertheless, it seems clear that a closure density ($\sim 10^{-26} \text{ kg m}^{-3}$) cannot be contributed by lenses with masses greater than $\sim 0.01 M_{\odot}$. Quasars simply have too small a scatter in line-to-continuum ratios and do not vary sufficiently. This argument needs to be strengthened, however: at present, densities of one-tenth of closure are not ruled out, and it has yet to be conclusively proved that the Universe is any denser than this on average. In any case, with the currently most popular candidate for the missing mass, the massive neutrino, having a mass of only $\sim 10^{-65} M_{\odot}$, the present lower limit of $\sim 10^{-2}$ is clearly capable of some improvement.

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100 years ago

THE FLORA OF ANCIENT EGYPT

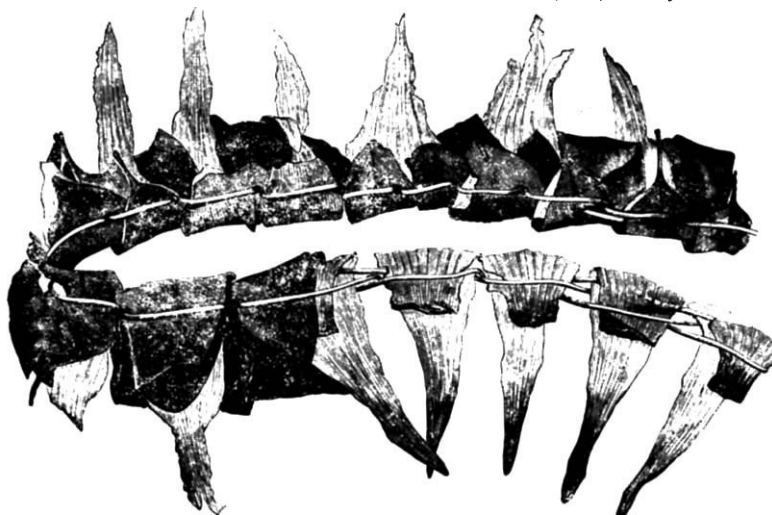
THE discovery made by Emil Brugsch Bey on July 6, 1881, in the vault of a king of the twentieth dynasty is of the greatest importance to botany in consequence of the large number of species of plants contained in the offerings and funeral repasts and in the wreaths which adorned the illustrious dead. Among them are several which were not known to belong to ancient Egypt.

The wreaths of Ramses II were renewed towards the end of the twentieth dynasty (1100 or 1200 BC), or at the time of the twenty-first dynasty (1000 BC). The king of that period caused a new coffin to be made for the great Ramses, the one in which he had first been placed having been accidentally destroyed. In this new coffin were several yards of wreaths.

The wreaths of Ramses II are formed of the

leaves of *Mimosa Schimperii*, Hochst., either folded or torn in two and stitched together, and serving as clasps for the sepals and petals of *Nymphaea coerulea*, Savi, and *Nymphaea Lotus*, Hook., the whole strung on strips of the leaves of the date palm. Besides the wreaths, there were in the coffin at the side of the body, and fastened between the bands encircling the mummy, whole flowers of *Nymphaea coerulea* on stalks eighteen or twenty inches long. The water-lilies thus scattered separately on the mummy were all of the blue-flowered species. An examination of these entire flowers and the sepals and petals in the wreaths leaves no doubt whatever respecting their identity with the living plants so common in ditches at the present day, especially in Lower Egypt, where they blossom from July to November.

From *Nature* **28**, 109, 31 May 1883.



Portion of a funeral wreath from the tomb of Ramses II.

Acquired immune deficiency syndrome

AIDS: the widening gyre

from Jerome E. Groopman and Michael S. Gottlieb

YEATS' vision of the apocalypse as an enlarging maelstrom, a widening gyre¹, inexorably dragging the world into destruction, has been re-created less poetically in a flurry of articles in the lay press on the epidemic of acquired immune deficiency syndrome (AIDS). Panic is inappropriate and indeed counterproductive in present circumstances. Yet faced with a striking and sustained increase in the incidence of AIDS in the United States, and its recognition in Europe, what has been done and what is to be done?

Based on data gathered by the Centers for Disease Control (CDC), there has been an exponential rise in the incidence of AIDS, with four to five new cases daily, a doubling of cases every 6 months and an estimate of 20,000 victims by 1985. The projected figures undoubtedly underestimate the extent of the epidemic since many cases are not reported to the CDC.

Moreover, CDC's surveillance definition of AIDS includes only opportunistic infections in previously well individuals or the occurrence of Kaposi's sarcoma under age 60 (ref. 2). Unknown numbers of men with prolonged unexplained lymphadenopathy and abnormalities of immune function are not reported to the CDC. This group is large and may represent a syndrome prodromal to AIDS; recent data indicate that up to 5–10 per cent of this group develop AIDS over a 12–15-month period³.

The risk of AIDS in certain census tracts of San Francisco has been estimated at 1 case per 350 males at risk⁴. Between 50 and 80 per cent of asymptomatic urban homosexual men⁵ and 20–30 per cent of haemophiliacs⁶ receiving factor VIII concentrate manifest 'T-cell imbalance' with a decreased ratio of phenotypic helper to suppressor/cytotoxic T lymphocytes. The cause of this imbalance is unknown and its relationship to AIDS undefined. While such perturbations are not specific for AIDS, it is likely that some instances of asymptomatic T-cell imbalance represent exposure to the aetiological agent of AIDS, given the increasing numbers of cases among these groups. Elevated levels of thymosin alpha-1 (a thymus hormone involved in the maturation of T cells) in a high percentage of AIDS patients and less frequently in asymptomatic individuals at high risk may be indicative of compensatory thymic response to end-organ (T-helper cell) depletion⁷.

The gyre has widened to include not only homosexual and bisexual men, haemophiliacs, intravenous drug addicts and Haitians but children⁸, central Africans⁹, female sexual partners of AIDS victims¹⁰ and random transfusion recipients as well. Case reports of opportunistic infections

among immigrants from Chad and Zaïre residing in Europe for months to several years appear identical with those of American AIDS. Opportunistic infections in heterosexual individuals who are not promiscuous and have not used intravenous drugs or received blood products are unexplained, and comprise about 6 per cent of all AIDS cases.

As with African immigrants to Europe, most Haitians who develop AIDS in the United States are heterosexual and have resided there for more than two years¹¹. Such occurrences imply that the latency from contracting the presumed AIDS agent(s) until appearance of opportunistic infection or neoplasia may be quite prolonged (up to several years) and may be a multi-factorial process.

AIDS has also occurred in individuals outside the high-risk groups after random blood transfusion with a latency of 6 months to a year, raising the issue in some cities whether the methods for screening of blood donors must be altered. The United States Public Health Service has recommended that blood products be used only when clearly indicated, that persons in high-risk groups for AIDS voluntarily defer blood donation and that autophlebotomy be performed before elective surgery so that a person's own blood can be used in the operating theatre if needed¹².

Many haemophilia centres are now limiting the use of factor VIII concentrate, a plasma product pooled from as many as 20,000 different donors, and are using instead cryoprecipitate, a product of greater volume derived from fewer donors. Since the putative agent of AIDS has not been identified, surrogate laboratory tests for mass screening of blood donors with AIDS are being investigated. So far, no single test or panel of tests has been validated for detecting individuals with clinical AIDS and certainly not for those incubating the disease.

Two primate centres in the United States have reported spontaneous outbreaks of opportunistic infections, lymphomas and fibrosarcomas occurring sporadically over the past 6 years^{13,14} but from the preliminary data it is not at all clear that this is a primate form of human AIDS (see ref. 15).

There has been some progress in further defining the immunological defects in AIDS. Although AIDS patients have markedly elevated serum immunoglobulin levels, humoral immunity appears disturbed. Certain AIDS patients failed to make humoral responses upon deliberate immunization¹⁶. Their paradoxical hypergammaglobulinaemia may be an epiphenomenon related to polyclonal B-cell stimulation following reactivation of latent

Epstein-Barr virus infection¹⁷.

Immunological reconstitution of AIDS lymphocytes *in vitro* has been attempted using a number of lymphokines including γ -interferon and interleukin 2 (ref. 18); both lymphocyte proliferative responses and HLA-restricted cytotoxicity against target cells infected with cytomegalovirus have been augmented using these lymphokines, and thus provide a theoretical basis for their use in man. Two clinical trials employing recombinant α_2 -interferon in the therapy of Kaposi's sarcoma associated with AIDS have recently been reported^{19,20}. Complete or significant partial remissions of Kaposi's sarcoma were achieved in about half of the treated patients, but there did not appear to be sustained augmentation of the patients' immune systems after α_2 -interferon.

Spontaneous recovery of T-cell numbers and functions in AIDS may rarely, if ever occur. It has been asked whether anything short of bone-marrow transplantation will be able to reconstitute the damaged immune system in AIDS. Two identical twin transplants have been performed without concurrent radiation or chemotherapy in patients with Kaposi's sarcoma and AIDS. Preliminary data from one recipient indicate a minor increment in his helper T-cell population but his clinical course was unchanged by the transplant²¹. Information derived from such procedures will certainly be important in determining whether normal lymphocyte stem cells can survive or be nurtured in hosts where there may be persistent infection with the causative AIDS agent.

Heightened public concern as well as changes in health care legislation in the United States will certainly affect patients with AIDS. The National Institutes of Health as well as private foundations are committed to increasing economic support for both clinical and research activities on AIDS, while both state and private insurance carriers have instituted cost capitation restrictions on health care. The AIDS epidemic will impose substantial economic stress on these new systems; the cost of clinical care for an AIDS patient is often \$50,000 to \$100,000 and the ultimate outcome fatal. One hopes that this economic burden will result in an even greater commitment to determining the aetiology and effective therapeutic modalities in this disease, and not result in decreased quality or quantity of care for such tragically afflicted people.

The key to effective action lies in finding the aetiology of AIDS. The epidemiological evidence leads to the virtually inescapable conclusion that a transmissible agent, presumably viral, is causally related to the epidemic. Such an agent may indeed have a prolonged incubation period, result in persistent viraemia and require a multi-factorial setting to lead to clinical AIDS. Because the human T-cell leukaemia virus (HTLV) has a tropism for helper T cells, it has been proposed that HTLV may be

aetiologically important in AIDS, a syndrome marked by helper T-cell depletion.

Several laboratories have now studied patients with AIDS for evidence of HTLV (refs 22–25 and *Nature, News and Views* 303, 377; 1983). Initial studies of M. Essex (Harvard University) demonstrate an increased prevalence among AIDS patients of antibody against HTLV cell-membrane antigens²⁴. R. Gallo (NCI) has convincingly shown integrated HTLV-like proviral sequences in DNA from fresh as well as cultured lymphocytes from two AIDS patients²³. It is not yet known which population of lymphocytes was infected with virus. Isolates of type-C lymphotropic retroviruses from one AIDS patient and one patient at high risk for AIDS appear similar but not identical to previous HTLV-I or HTLV-II isolates^{22,25}. One might postulate that such retroviruses share sufficient homology with HTLV-I to be detected by immunofluorescence and hybridization techniques yet act as lytic retroviruses resulting in destruction of the T-helper cell population.

While these recent data are intriguing, HTLV infection in AIDS patients may simply be an epiphenomenon related to their profound immune deficiency and increased susceptibility to a variety of viral infections. In addition, transformation *in vitro* with HTLV has largely been accomplished using co-cultivation techniques, implying that HTLV infection may require cell-cell contact. One would have to postulate that an 'AIDS-HTLV' could be transmitted by cell-free vectors such as factor VIII concentrate. On the other hand, the involvement of an HTLV-like virus in the pathogenesis of AIDS would provide support for a two-virus model of the genesis of Kaposi's sarcoma in AIDS. Cytomegalovirus has been associated with Kaposi's sarcoma in culturally diverse populations and could be oncogenic in the milieu of immune deficiency created by the AIDS agent. Since homosexual males have a markedly higher carriage rate of cytomegalovirus compared with heterosexual

controls²⁶, a two-virus model could explain the preponderance of opportunistic infection alone among heterosexual AIDS patients (90 per cent) compared with the prevalence of Kaposi's sarcoma among homosexual AIDS patients (50 per cent).

The widening gyre of AIDS has begun to encompass the attention of increasing numbers of virologists, immunologists, epidemiologists and clinical investigators

throughout the world. Considering the exponential increase in incidence and sustained fatality rate, one hopes that their concerted efforts will yield important aetiological and therapeutic information in the near future. □

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Earthquake mechanisms

Seismicity at Mammoth Lakes

from Leon Knopoff

THE popular ski and winter sports community of Mammoth Lakes, California is located at the south-west edge of Long Valley caldera, a roughly elliptical region 17 × 33 km in dimension with its long axis pointing east-west. The caldera was created in an enormous eruption 700,000 years ago that released about 600 km³ of ash, a volume more than 600 times larger than that released in the Mt St Helens eruption. Since 1978, seismicity in the caldera has increased, with activity culminating in a series of four earthquakes of magnitudes 5.6–6.1 within a period of 48 hours during 25–27 May 1980. Several earthquake swarms have subsequently occurred in the caldera, including more than 1,000 earthquakes of magnitude 1 or greater on 6 January 1983. These seismic events are remarkable for two main reasons. The first is that activity of such magnitude is quite unprecedented in the region. The second is that, according to a report by Bruce Julian in a recent issue of *Nature* (303, 323; 1983), the events may be the first recorded examples of an earthquake mechanism that has until now been regarded as no more than a theoretical possibility.

A particularly interesting feature of the Mammoth Lakes seismic activity is that a large number of first motions of events in the 1980 earthquake swarm were inconsistent with the radiation pattern expected from a double-couple model for the earthquake focal mechanism. In his recent paper in *Nature*, Bruce Julian showed that these discrepancies can be minimized by using a compensated linear vector dipole (CLVD), a mechanism which had been proposed theoretically before but never observed in practise.

It has long been known that the double-couple mechanism only uses two of the five degrees of freedom available for source focal mechanisms that radiate in the second order of spherical harmonics. Second-order spherical harmonic source mechanisms are the terms of lowest order that have neither a net force nor torque, a requisite condition for equilibrium of an isolated body such as the Earth. Double couples are the force equivalents for shear fracture on a plane. The remaining three degrees of freedom correspond, non-

uniquely of course, to sudden tensile fractures on a plane; the motions are those of two planes that move in directions parallel to the normals and oppositely directed. The force vectors associated with these motions are colinear and oppositely directed. Effects of volume changes associated with this form of relative motion can be easily compensated.

Oppositely directed tensile motions on a fracture plane require much more energy than shear fractures on a plane. So it is not surprising that it is predominantly the double couple that has been observed to date. Indeed the events in Long Valley caldera had been interpreted on this basis, largely as a matter of custom. Thus Julian's demonstration that these are of the CLVD type elevates this focal mechanism out of the realm of theoretical curiosity.

Julian concludes that it is most likely that the fractures were due to the injection of fluid, probably magmas, into a pre-existing fissure which subsequently expanded rapidly. Thus the large earthquakes of three years ago probably were important signatures of the upward migration of magma under the region.

Various independent geophysical observations have indicated the rise of magma beneath the Long Valley caldera. Earthquake epicentres rose from a depth of 9 km in 1980 to within 3.5 km of the surface by 1982. The earthquakes have occurred spasmodically, a feature of pre-eruption volcanic histories, and increased fumarolic activity about 3 km from the epicentre has been noted. Moreover, releveling of the region showed that the dome in the western part of the caldera had bulged upwards about 25 cm perhaps between July 1979 and October 1980. A magma chamber was known to lie under this region between depths of 8 and 15 km, so it was speculated that magma had moved within the chamber. Indeed, in May 1982, the US Geological Survey issued a notice of potential volcanic hazard for the region. This notice is the lowest of three levels of concern about possible future volcanic activity. □

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Ethiopian flood basalt province

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The most voluminous outpourings of lava on the land surface of the Earth have built imposing stacks of superimposed basalt flows, as much as several thousands of metres thick. Such stacks, or piles, were fed from linear zones of fracturing and fissuring in the crust through which basaltic magma emerged in successive pulses, flooding out over the surrounding region for distances as far as a few hundred kilometres. The fluid nature of these 'flood basalt' lavas resulted in the formation of a subdued or planar new topography which, when uplifted during subsequent tectonic episodes, has produced what are popularly termed 'volcanic plateaus'.

IGNEOUS provinces, comprised largely or almost entirely of flood basalts, are preserved in the Keweenaw district of north-central USA (Proterozoic), the central sector of the Siberian platform (early Mesozoic), the Karroo and neighbouring areas of South Africa (Jurassic), the Paraná plateau of southern Brazil–Uruguay and the Deccan plateau of western India (both Cretaceous), and the mid-Tertiary plateaus of Columbia River (western USA) and Ethiopia–Yemen. The larger of these provinces cover original areas of $\sim 1 \times 10^6 \text{ km}^2$. Individual flows are typically 10–30 m thick, and a maximum of some hundreds of flows in the central sector of a province can lead to a total volume exceeding $0.5 \times 10^6 \text{ km}^3$.

Flood basalt eruptions have, in most instances, been intimately associated with initiation and early development of rifted continental margins (Fig. 1). Field studies have led to the recognition^{1,2} of a relationship between: (1) tensional faulting associated with crustal attenuation and warping; (2) feeder-dyke swarms representing, after erosion, the original surface fissures from which the lavas emerged; and (3) the flood basalt pile. The Ethiopian flood basalt province is one of several that

became active during formation of new continental margins, born with the Mesozoic–Cenozoic fragmentation of Pangaea. Although volcanological studies remain in their infancy, the Ethiopian province offers an unusually challenging insight into the development of a volcanic continental margin, and into the mantle processes that underlie the generation of new, quasi-oceanic crust there.

Location and extent of Ethiopian lava pile

The Ethiopian highlands were early recognized and mapped as a flood basalt province³, and later in the nineteenth century Blanford⁴ (the first to recognize the existence of pre-Pleistocene glaciations, in India) noted that "the enormous mass and persistent horizontality [of the Abyssinian and Deccan lavas] over large areas, entitle them to much greater attention than they have received". Attention followed^{5–12}, but not through English-language accounts (except ref. 12) until the 1960s.

The Ethiopian volcanic province is dominated by a Tertiary basaltic pile, fed from fissures aligned along developing pairs of opposed, nascent continental margins. The Ethiopian and Yemen margins run either side of the Red Sea, and within Ethiopia the plateau margins face each other across southern Afar and the rift valley (Fig. 2). In detail, it is seen that in northern Ethiopia the dyke-swarms turn oblique to the Afar-plateau margin, around the northern end of the thicker part of the basalt pile—Figs 2 and 3—refs 13, 14. In Ethiopia, the development of volcanic continental margins is complicated by association with a triple-rift junction¹⁵ where spreading rates

Table 1 Relative proportions of mildly alkaline and tholeiitic basalts in various Ethiopian stratigraphical sequences, based on numbers of chemically analysed specimens⁹¹

	A/A + T
Southeastern Plateau flood basalts:	
northern sector (61 analyses)	0.30
southern sector (26)	0.95
Western Plateau flood basalts:	
northern sector—Termaber Formation (66)	0.92
Alaji Formation (45)	0.20
Aiba Formation (38)	0.05
Ashanghi Formation (36)	0.46
southern sector (36)	0.95
Tana basin fissure basalts:	
Quaternary (11)	0.72
Tertiary (11)	0.45
Afar stratoid flood basalts (30)	0.44
Danakil block fissure basalts (68)	0.87
Rift valley fissure basalts:	
axial Wonji fault belt (23)	0.93
off-axis (4)	1.0

A, No. of specimens passing two tests⁹¹ for alkaline basalts; T, no. of specimens answering the same two tests as subalkaline (tholeiitic).

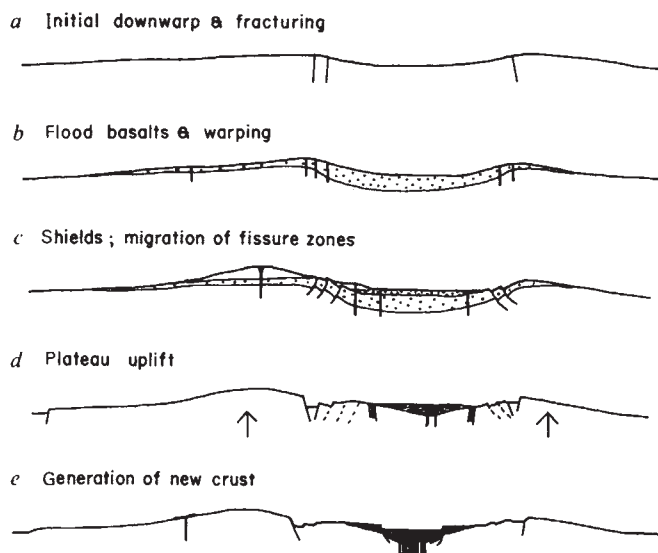


Fig. 1 Cross-section pictorials of the evolution of the Ethiopian flood basalt province. Note with *a*, broad proto-rift depression; *b*, parallel sets of fissure-feeders are active at basin margins, accompanied and followed by antithetic faulting as crust thins under tension; *c*, sites of active fissure-feeders migrate into basin; *d*, differential uplift at site of first major volcanism and faulting, and second development of such volcanism and faulting in centre of basin; *e*, basin widens as new basaltic crust is emplaced in response to ongoing tension.

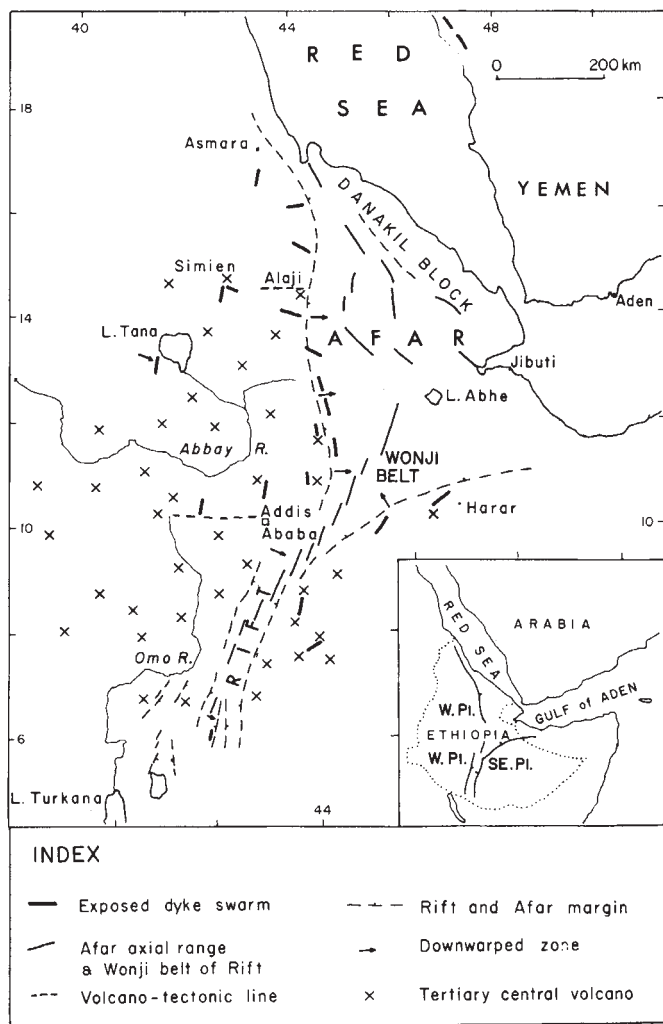


Fig. 2 Known volcanic sources in the Ethiopian region, and their relationships to plateau and Afar/rift valley tectonic domains. (W.PI. = Western Plateau, SE.PI. = Southeastern Plateau.)

are slow (half-spreading rate $<1 \text{ cm yr}^{-1}$). Because of this, the normal process of crustal warping and progressive subsidence through sea level has been inhibited by a persistent proximity of Pliocene–Quaternary rift floor volcanism^{16–18} and high geothermal gradients¹⁹ close to the Tertiary continental margins. Thus the Afar depression (Fig. 2) substitutes for a new ocean basin.

The area covered by Tertiary flood basalts in Ethiopia, excluding the once contiguous Yemen province, is $\sim 600,000 \text{ km}^2$. An area close to $750,000 \text{ km}^2$ must have existed before plateau uplift and ensuing massive erosion occurred during Pleistocene time^{20–23}. The volcanics are distributed asymmetrically about Afar and the rift valley, being volumetrically biased to the western side (Fig. 3). The asymmetry faithfully matches that of the subsequently uplifted Ethiopian highlands, comprising the broad Western (Ethiopian) and narrow Southeastern (Harar or Somalian) plateaus to either side of the rift valley. Volcanism's role as one surface manifestation of continuing thermal expansion of the upper mantle is thereby emphasized.

The sites of magmatic egress in Ethiopia are imperfectly known. Despite the incision of dramatic river canyons into the plateaus, deep erosion has not yet advanced as far as the plateau rims overlooking Afar and the rift valley. These are precisely the expected loci of the fissure-feeders for the lavas, aligned along the zone of maximum crustal warping (Figs 1 and 2). However, reconnaissance surveys have revealed major dyke swarms poorly exposed within and, in most cases, parallel to the western and southern margin zones of Afar^{9,13}. Other fissure

loci must occur in the interior of the Western Plateau, to account for the extent of the flood basalts as far as the Sudan border region, some 400–500 km west from the Afar and rift valley margins. One such appropriately located, north–south trending dyke swarm has been described from the Guder Canyon, 120 km west of Addis Ababa and the rift margin¹³.

The problem of precisely defining the Ethiopian flood basalt province is illustrated in the first instance by the superposition, on the fissure-fed lavas, of locally thick sequences (exceeding 1,000 m) of basalt flows that emanated from central volcanoes. These volcanoes built coalescing, low-angled shields, exemplified by the Miocene Simien centre^{24,25}. An absence of regional unconformities suggests a gradual passage during Miocene evolution from fissure to more-focused central eruptions in Ethiopia. Because of the present impossibility of identifying, in the field, any boundary between true flood basalts and shield basalts, the latter are also discussed in this review.

The Ethiopian volcanic province remains active²⁶. This adds a further difficulty to defining the upper age limit of the flood basalts. Although the youngest basalts of regional extent on the plateaus are those forming the Miocene shields, volcanism has continued since then, but from sites progressively further into Afar and the rift valley²⁶. Late Tertiary and Pleistocene volcanism, now concentrated within and immediately fringing Afar and the rift valley, has included both fissure and central eruptions. The Afar stratoid basalts (Fig. 3) are the most important example of the former, flooding most of central and southern Afar during late Pliocene–early Pleistocene time^{27–29}. If in the future Afar divides, due to continued drift of Arabia away from Africa, then the stratoid basalts will mantle the new pair of continental margins in the same way as the earlier flood basalts mantle the Tertiary margins of the Ethiopian and Yemeni plateaus. It would appear unwise, therefore, to ignore the Pliocene–Quaternary volcanism in a study of the Ethiopian flood basalts, even though the proportion of silicic volcanic products has dramatically increased with time^{20,23,30}.

During the past 1.0 Myr, volcanism in Afar has focused into discrete 'axial volcanic ranges'^{28–30} (Fig. 2). During the same time, the rift valley has been strongly downfaulted relative to the plateaus to either side. Immediately preceding this structural evolution, there was massive eruption of silicic ignimbrites from calderas situated both outside and within the present confines of the rift valley³¹. Since 1.0 Myr, however, both basaltic and silicic eruptions have been almost entirely restricted to the axial Wonji fault belt of the rift floor^{24,32} (Fig. 2). Interesting but volumetrically minor basalt eruptions have occurred on both plateaus of Ethiopia during Quaternary time, notably in the Tana basin and along fault zones extending westward from the Addis Ababa region^{11,23,33}.

Despite the sporadic peppering of the plateaus by Quaternary basalts, the general picture is of one of a narrowing of the zone of Ethiopian rift volcanism and tectonism with time³⁴, as in Kenya³⁵. It is logical to relate this to a progressive thinning and necking of the lithosphere below the African rifts^{24,36}. The asymmetric position of the necked zone with respect to the Ethiopian uplift and its volcanic cover (Fig. 3) has persisted for at least the past 15 Myr³⁷. Whether Tertiary basaltic volcanism along the eastern margin zone³⁸ of the rift–Afar depression was less voluminous than along the western margin³⁹ remains uncertain, as flow direction may have been biased by topographical factors⁴⁰. South-west from Afar, the fault-pattern defines numerous right *en echelon* transpositions of the rift valley, and reduces what would otherwise be an even grosser asymmetric transect of the Ethiopian uplift and its flood basalt capping. This confirms that volcanism, rifting and uplift are linked, though not necessarily coeval processes^{22,31,41}.

The total volume of the preserved Ethiopian Cenozoic volcanics has been estimated at $\sim 350,000 \text{ km}^3$, of which $300,000 \text{ km}^3$ are of basaltic composition⁴². The unknown volume of basalts beneath Afar and the rift valley is not included in this estimate, which is therefore a minimum. Although the basalt pile typically thickens from periphery to centre of the

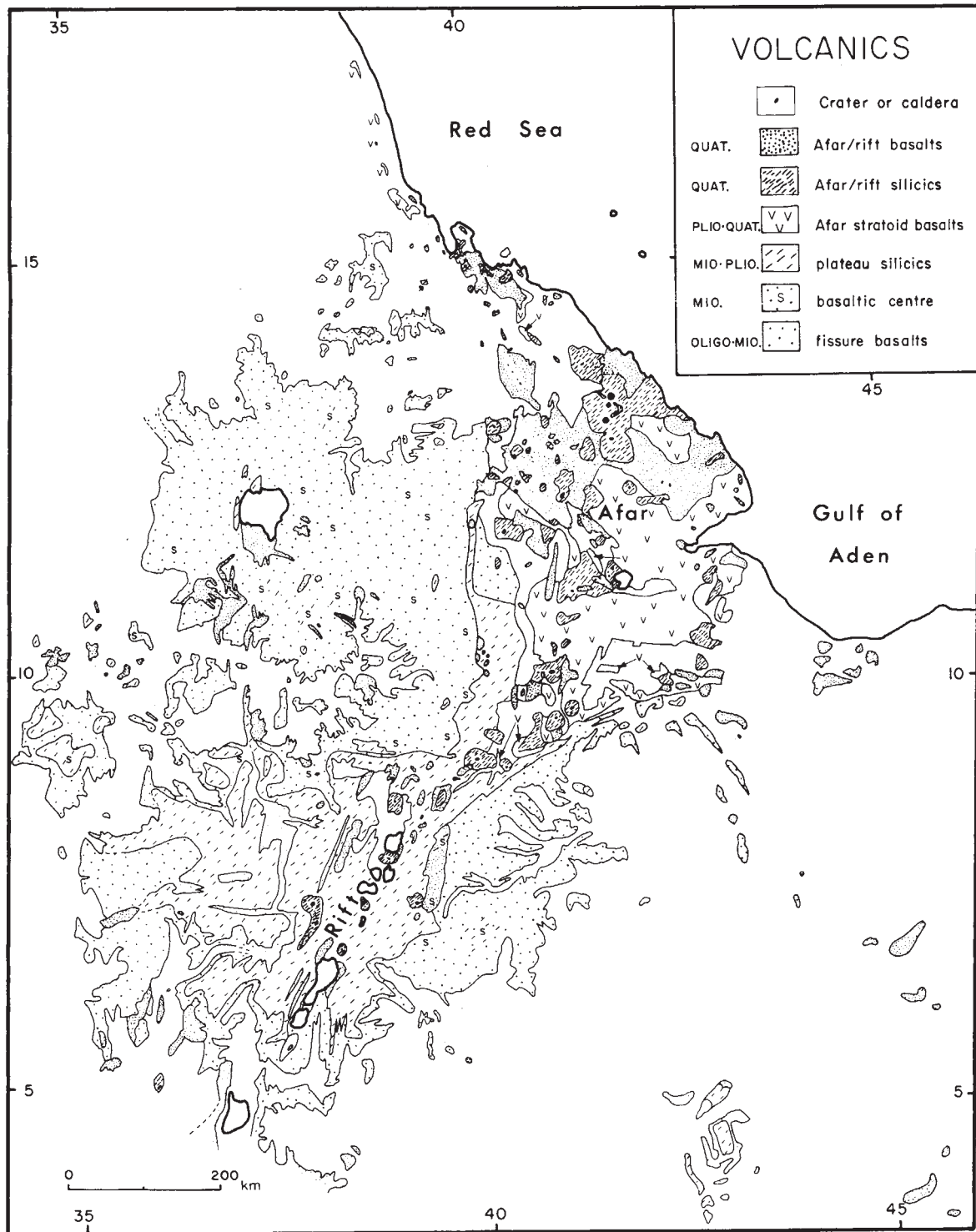


Fig. 3 Simplified volcanological map of Ethiopia.

Ethiopian uplift¹⁰, where values of 1,000–2,000 m are reached in the zones of feeder dyke swarms⁴³, there are several instances where lateral thickening is abrupt across poorly-studied tectonic lines within the plateaus. Thus the east–west trending Alaji and Addis Ababa fault zones^{8,42,43} and the north–west–south–east Marda lineament passing east of Harar^{44,45}, align eruptive sites that, although minor in comparison with the fissure zones of the Afar and rift valley margins, may well have erected palaeotopographical boundaries which controlled lava flowage and ponding⁴⁰.

Stratigraphy

Blanford's⁴ original classification of the northern Ethiopian volcanics retains valid elements, in particular the distinction

between an older Trap Series on the plateau and a younger Aden (or Rift⁴⁶) Series in Afar. Zanettin *et al.*⁴⁶ place the boundary between the two series at ~14 Myr, close to the age postulated for the initiation of the Ethiopian³⁷ and Kenyan⁴¹ rift valleys. However, Blanford's division of the Trap Series into earlier, Ashanghi basalts overlain by Magdala basalts was based on the observation of a profound but, it is now realized, local unconformity within the flood basalt sequence south of Alaji^{4,39}. Attempts to extend Blanford's lithological criteria for distinguishing the two basalt formations to the Ethiopian region as a whole^{47,48} have been unsuccessful¹⁰.

Despite deep erosional dissection of the basalt pile on the plateaus, the resulting canyon wall exposures are not easily accessible for field studies. This has inhibited an understanding

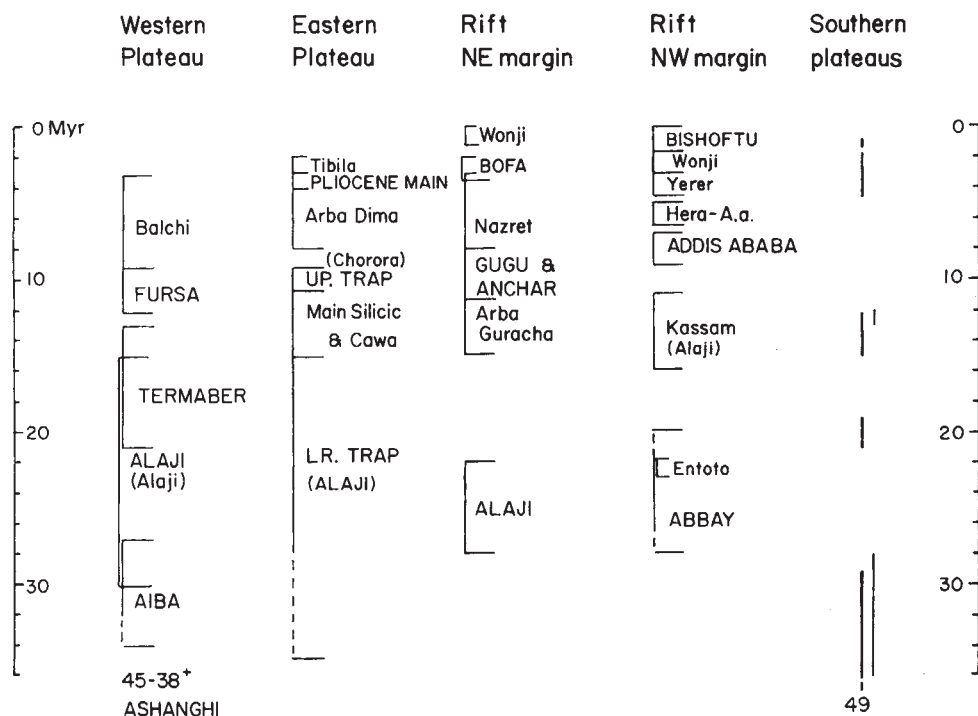


Fig. 4 Volcanic formations of the northern sectors of the two plateaus, and the plateau-rift margins^{38,55-58}. Formations in which basaltic rocks dominate are written in capital letters. The southern sectors of the two plateaus remain sketchily known^{33,63}, and only age-ranges are presented (thick line, basaltic; thin line, silicic rocks). For discussion of age of Ashangi Formation basalts, see text.

of the stratigraphy and structure of the pile to the extent that, on the most recent geological map of Ethiopia²³, the stratigraphy has been extrapolated horizontally for hundreds of kilometres from the few localities where it has actually been studied. This extrapolation brings about some contradictions with other field observations and also with radiometric age-data; one important contributing factor is surely the existence of volcanologically distinct sub-sectors along a given fissure/dyke swarm zone, as well as of the individual, albeit coalescing basaltic shields referred to above⁴⁹.

The most intensive stratigraphical studies to date have been made along the rim of the Western Plateau overlooking Afar. Lava samples collected up convenient altimetric traverses have been dated by the K-Ar method⁵⁰⁻⁵⁴. This approach, combined with broad lithological identifications, has provided for a new classification of the volcanic succession on the Western Plateau bordering Afar⁵⁵⁻⁵⁸ (Fig. 4). A similar approach has been followed in providing a new stratigraphy for the northern sector of the Southeastern Plateau, again where bordering Afar^{38,59} (Fig. 4). The ambitious scope of the new work on the Western Plateau does, however, carry inherent disadvantages: (1) no type sections have been described where formation boundaries can be demonstrated; (2) the lateral behaviour of individual or groups of flows has not been followed, so that the structure of the basalt pile remains unelucidated; (3) the resulting looseness of the scheme has encouraged its premature application to other sectors of the plateaus wherever K-Ar apparent-ages become available; and (4) the accuracy of some crucial radiometric dates is disputed (see ref. 46), while some others have already either been revised or rejected⁵⁴.

The stratigraphies of the volcanic pile at various localities or sectors in Ethiopia are summarized in Fig. 4. Formation thicknesses can vary greatly along strike^{10,20} and, for the flood basalts comprising the Ashangi, Aiba and Alaji formations, increase towards the rift valley and Afar, where the base of the pile is generally not exposed. For example, in the Addis Ababa region the flood basalts, variously ascribed to the Aiba⁵⁶, Aiba and Alaji⁴⁶, and Ashangi + Aiba + Alaji⁶⁰ formations, increase in thickness eastwards from 250 m in the Abbay basin to a minimum of 2,000 m north of Addis Ababa^{20,22,61}. A similar pattern is seen in cross-sections of the pile across the Southeastern Plateau-Afar margin^{38,59}. Thickening is accomplished by a combination of increased numbers of flows, and increasing thickness of individual flows. The thickest flows reach

40 m, but 20 m is a more typical thickness for the plateau flows.

The basal, Ashangi formation presents an enigma. Merla and Minucci⁸, after mapping the type-area, noted that nowhere could the existence of Blanford's unconformity at the top of the Ashangi basalts be conclusively demonstrated. The same authors identified intense faulting in this region, of both compressional and extensional nature, later confirmed by other workers⁴³. However, Zanettin and co-workers claim that the unconformity does exist, and ascribe their radiometric age-data on the Ashangi basalts, in the range 30-24 Myr and coeval with or slightly younger than their age-data for the overlying Aiba basalts (Fig. 4), to the tectonic deformation of and isotopic resetting in the Ashangi basalts³⁹. The same authors therefore ascribe an Eocene age to the Ashangi formation, variously 60-45 Myr (ref. 60) and 45-38 (ref. 46). They also extend the occurrence of the Ashangi basalts south of the type-area as far as the latitude of Addis Ababa⁶⁰, where, however, no unconformity is witnessed within the flood basalt sequence. It is prudent not to exclude the possibility that, in the type-area, the Ashangi basalts are tilted and locally overthrust basalts, coeval with the Aiba formation, notwithstanding mineralogical and geochemical distinctions^{45,57,60}. This would leave only the oldest basalts of southwestern Ethiopia³³ as being of Eocene age.

The Aiba basalts represent the greater part of the flood basalt pile over the northern part of the Western Plateau. The base of the overlying Alaji formation is defined by the first rhyolitic ignimbrite, but where such ignimbrites are absent, no distinction between the two formations can be made on either lithological or geochemical grounds^{46,60}. This difficulty is compounded by a strong diachronism in the earliest rhyolitic volcanism between the latitudes of Alaji and Addis Ababa^{46,51}, and accounts for the formation overlaps summarized in Fig. 4, column 1. In fact, the catalogue of radiometric ages published by Merla *et al.*²³ reveals, not progressive diachronism but separate 32-28 Myr and 18-15 Myr rhyolitic episodes, respectively in the Alaji and Addis Ababa sectors. The disparity between the age-ranges, plus the local occurrence of 23-22 Myr-old rhyolites at Addis Ababa itself, suggests that the Alaji formation is in need of redefinition, perhaps along the lines originally proposed by Justin-Visentin and co-workers^{51,55,56}.

Where the preserved pile is composed entirely of basaltic rocks, correlations are at present restricted to radiometric con-

siderations and doubts become compounded. Thus the flood basalts of the Abbay Canyon, 125 km north-west of Addis Ababa, have been ascribed to the Aiba basalts on the basis of an apparent 31–28 Myr age-range^{50,55}. But other workers have obtained age-ranges of 27–23 Myr (ref. 22) and 22–19 Myr (refs 52, 53), and on reviewing these additional data, Zanettin *et al.*⁴⁶ have reclassified the Abbay succession as comprising both Aiba and Alaji basalts. It is evident that the remedy for the present unsatisfactory state of the stratigraphy of the Ethiopian flood basalts^{23,47}, even in the type areas of the Western Plateau, is a focused and thorough mapping programme that will require helicopter support to survey canyon walls.

Early rhyolites intercalated in the flood basalt pile occur not only in the northern part of the Western Plateau, but also on the southern fringes of the same plateau in south-west Ethiopia, extending east as far as the future site of the rift valley^{33,62,63}. By contrast the younger, mid-Miocene rhyolites were associated with on-going development of the Afar margins^{17,27,64–73} and initiation of the rift valley^{37,46}.

The Termaber basalts⁴⁶ correspond to the shield basalts superimposed on the fissure flood basalts, and represent the last major volcanic episode on the plateaus. These shields fall into two known age-ranges: 26–22 Myr on the northern part of the Western Plateau, and 15–13 Myr on the central part and the Southeastern Plateau^{25,46,51,66}. Again, as for the Alaji basalts, there is a need for a re-assessment of the term Termaber.

The preceding discussion has concentrated on the stratigraphy of the basalt pile of the Western Plateau where this plateau borders Afar. This is where logistics have constrained most work to be done up till now. By contrast, the stratigraphy of the basalts further west towards Sudan, and on the southern halves of the two plateaus, remains mostly unexplored^{62,63,74,75}. Furthermore, the stratigraphy of the Pliocene–Pleistocene flood basalts of Afar has not been discussed: dissection into this low-lying lava pile has been negligible, with less than 300 m of the top of the stratigraphy exposed^{28,76}.

Alkaline or tholeiitic basaltic province?

The results of pre-1960 petrographic studies on Ethiopian basalts^{6–11,77–80} led to reviews that claimed an alkaline character for this volcanic province^{48,81–83}. Gass^{81,82} further proposed a temporal evolutionary sequence from alkaline through transitional to tholeiitic basalt activity, characterising respectively the present plateaus, Afar and Red Sea floor regions. The trend of decreasing basalt alkalinity with time was ascribed to shallower mantle melting as the lithosphere became progressively more attenuated^{41,81}. However, geochemical researches during the past decade require this model to be modified.

The nomenclature of Ethiopian basalts is not yet standardized. Both mineralogical and chemical criteria have been used^{11,14,79,84–88}, but emphasis has tended to be put on the latter. The distinction between alkaline and tholeiitic Ethiopian basalts has commonly been based on the alkalis–silica relationship obtained by Macdonald and Katsura from Hawaiian basalts⁸⁹. More recently, the term transitional basalt has been introduced, initially for some Afar Quaternary basalts⁸⁴, to describe rocks with an alkaline character that is chemically intermediate between that of alkali basalts and ocean floor tholeiites. The term has since been quantified on the Macdonald–Katsura plot to embrace basalts that fall within ± 1 wt% alkalis from the alkaline basalt–tholeiite dividing line. Piccirillo and co-workers^{46,60} note that the upper bound to this transitional basalt field corresponds closely with an alkalis–silica line separating fissure from central-derived Ethiopian plateau basalts. There is only partial correspondence of the transitional basalts thus defined with the olivine tholeiite field defined from CIPW normative mineralogy^{60,90,91}.

The application of electron microprobe analytical techniques to the mineral chemistry of Ethiopian and Kenyan basalts^{60,88,92} has led to mineralogical classification, based on established

criteria for distinguishing alkali olivine basalts from tholeiites^{48,84,90}, and includes the difficult discernment of a third, transitional type of mineralogy⁶⁰. In this review, distinction between alkaline and tholeiitic basalt is based on Chayes' revisions of alkalis–silica and normative mineralogical criteria^{91,93}. These two basalt types are here only separated and supplemented by a transitional type where previous work firmly establishes the close association in the field of three basalt types.

The recognition that tholeiitic basalts occur within the plateau lava sequence^{79,94} has now been extended to include almost the entire lower part of the pile on the northern sectors of the two plateaus^{57,95,96}. Only the upper part of this pile, derived from Miocene shield volcanoes, is dominated by alkaline basalts^{48,57} (Tables 1 and 2) that can attain a basanitic character. On the southern sectors of the plateaus, however, scanty data suggest that tholeiitic basalts are much less abundant than to the north (Table 1). Therefore it is with the development of the Afar margins^{29,54,59}, during late Oligocene–early Miocene, that tholeiitic magmas were voluminously erupted. Away from Afar and further into continental Africa, coeval flood basalts had a mildly alkaline character^{62,63}. Later in Miocene time, central volcanoes built up shields of alkaline basalt over much of the present plateau surface, as the rate of magmatic egress diminished.

In the preceding discussion, the term basalt has been used *sensu lato* to embrace more evolved rocks. In fact the flood basalts of the northern sectors of the plateaus can include up to 50% by volume of hawaiite/basaltic icelandite, though ~5% is more typical. Some basanitic shields have minor occurrences of associated phonolite^{57,97}. On the southern sectors of the plateaus, alkaline basaltic shields show a preponderance of hawaiite over basalt^{57,98}.

In Afar and the rift valley, basalts of tholeiitic, transitional and alkaline composition have been erupted during Pliocene–

Table 2 Average compositions of basalts and related rocks from Ethiopia; from northern half of Western Plateau unless otherwise stated

Wt%	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
SiO ₂	46.9	50.8	50.4	50.1	50.1	47.2	46.1	49.2	48.7
TiO ₂	4.8	3.3	2.7	2.8	3.3	2.7	3.0	2.1	2.9
Al ₂ O ₃	10.9	13.8	14.5	14.7	14.3	15.1	14.8	17.0	15.4
Fe ₂ O ₃	6.2	4.4	4.0	4.3	4.0	5.9	4.9	4.5	—
FeO	8.5	7.9	8.2	8.3	9.1	6.8	8.3	7.0	11.9
MnO	0.23	0.19	0.19	0.20	0.24	0.21	0.20	0.18	—
MgO	7.3	5.6	5.7	5.3	5.0	6.5	7.0	4.7	6.4
CaO	10.9	9.3	9.9	10.3	9.1	10.5	10.5	8.6	10.6
Na ₂ O	2.5	3.0	3.0	3.0	3.2	3.3	3.3	4.3	3.0
K ₂ O	1.2	1.0	1.0	0.69	1.2	1.3	1.1	1.6	0.63
P ₂ O ₅	0.59	0.56	0.51	0.34	0.46	0.62	0.82	0.82	0.50
Norm %									
q	0.7	4.2	2.0	1.9	1.7	—	—	—	—
or	7.0	6.0	5.8	4.1	7.2	7.6	6.6	9.6	3.7
ab	21.2	25.6	25.0	25.8	26.9	23.5	22.6	31.0	25.4
an	15.0	21.3	23.5	24.4	21.2	22.5	22.4	22.5	26.6
ne	—	—	—	—	—	2.6	2.9	3.0	—
di	28.5	17.7	18.7	20.3	17.2	21.3	20.4	12.8	19.0
hy	9.0	11.7	13.3	12.1	12.7	—	—	—	7.2
ol	—	—	—	—	—	10.8	11.9	11.1	9.4
mt	8.4	6.2	5.4	5.5	5.8	5.4	5.8	4.4	2.2
il	9.0	6.2	5.1	5.2	6.3	5.1	5.6	3.9	5.5
ap	1.3	1.2	1.1	0.7	1.0	1.4	1.8	1.8	1.1
MF	0.85	0.75	0.69	0.70	0.66	0.70	0.72	0.61	0.61
Type	TB	AT	AT	AT	AT	AB	AB	HA	TB

Columns: (1), 10 Ashanghi Formation basalts; (2), 33 Aiba Formation basalts; (3), 26 Alaji Sirro basalts; of 'Oligocene' age from Alaji sector⁵⁷; (4), 10 Southeastern Plateau flood basalts¹¹²; (5), 12 Alaji Molale basalts; of 'Miocene' age from north of Addis Ababa⁷⁷; (6), 23 Termaber Guassa basalts: older, more northerly shields; (7) 18 Termaber Megheze basalts: younger, more southerly shields; (8) 7 Bali hawaiites, southern Southeastern Plateau⁵⁷; (9) 11 Afar Stratoid Series basalts¹⁰².

MF = MgO/MgO + FeO in normative feric minerals.

Types: TB, transitional mildly alkaline basalt; AT, andesine tholeiite; AB, alkali basalt; HA, hawaiite; q, quartz; or, orthoclase; ab, albite; an, anorthite; ne, nepheline; di, diopside; hy, hypersthene; ol, olivine; mt, magnetite; il, illite; ap, apatite. The data have been drawn from the author's file of all published Ethiopian analyses, but are mainly from ref. 57.

Calculation of weight norms is based on % Fe₂O₃ not allowed to exceed % (TiO₂ + 1). This takes into account the observation that % Fe₂O₃ tends to be higher in the more alkaline Ethiopian basalts¹²¹.

Quaternary time, often in close proximity to one another^{28,85}. Afar basalts fed from fissures along strike with nearby central volcanoes can show stronger tholeiitic tendencies than basalts from isolated fissure systems^{28,99-101}. In the rift valley, basalts with a tholeiitic chemistry appear to be restricted to the axial Wonji fault belt^{31,32}, again in proximity to Pleistocene central volcanoes aligned along that belt²⁴. Basalts erupted elsewhere on the rift floor have a clearly alkaline character (Table 1). The near-contemporaneous generation of tholeiitic and alkaline basalts under the Afar and rift depressions suggests the persistence of a wide range of melting depths and/or degrees of mantle melting.

Piccirillo *et al.*⁶⁰ consider that control on the alkalinity of Ethiopian basalts stems essentially from the intensity of the lithospheric dilatation that accompanied magmatic egress. Strong dilatation would have facilitated a more voluminous emission of basalts of correspondingly more tholeiitic character. This is proposed to be well expressed in the massive tholeiite emission that accompanied initiation and early development of the Afar margins⁶⁰. A second voluminous episode, this time of basalts of predominantly transitional alkalinity^{28,102}, flooded Afar and the northern sector of the rift valley in Pliocene time. Here again there has been a relation to strong crustal extension, though most of the original feeder-fissures lie buried beneath an undissected cover of lavas and young sediments^{68,103,104}. By contrast, where and when dilatation was weak, alkaline basaltic activity predominated.

The thesis of Piccirillo *et al.*⁶⁰ for the genesis of the Ethiopian volcanic province leads to the interesting conclusion of a first-order independence of magma composition and volume from tectonic regime. The latter can be expressed in terms of crustal thickness and type, whether fully continental, attenuated continental, or oceanic. Thus the great floods of Aiba Formation basalts emanated through attenuating continental crust⁵⁴, but some also through unattenuated crust on the plateau, as did the Termaber shield basalts. By contrast, the Afar stratoid basalts have been erupted through neo-oceanic crust^{28,29}. Unfortunately, data remain too sparse to approach the next question, whether there may have been substantial disharmonic thinning of the lithospheric mantle beneath a fractured but unthinned crustal lid, such that there is no necessary relationship between crustal and lithospheric thicknesses. Moho isobath maps, derived from seismic refraction and gravity data, are available for Afar and environs¹⁰⁵, but estimates of lithospheric thicknesses are speculative and Tertiary lithospheric thicknesses remain unknown²⁴.

Magma genesis

From the numerous recent data and interpretations concerning Ethiopian magmatic sources and magma evolution, only a few selected topics can be alluded to here. Cox¹⁰⁶ has argued for the derivation of flood basalts from a picritic parental magma, by means of fractionation in sill-like magma chambers at or near the base of the continental crust. Consistent with this thesis, olivine, clinopyroxene, plagioclase and iron-oxide phenocrysts co-exist in many Ethiopian basalts^{60,79,80,86} both from plateaus, and Afar and rift. Plagioclase and olivine-augite basalts are not uncommon, especially among the plateau lavas^{7,11,79}. Although aphyric rocks can predominate among the more tholeiitic basalts of both plateau⁶⁰ and Afar⁸⁷, positive mineralogical evidence for near-cotectic crystallization and low-pressure equilibrium exists for the greater part of the Ethiopian basalts, the more strongly alkaline rocks providing a prominent exception. Polybaric fractionation has been invoked to explain the mineral chemistry of spinel harzburgite and olivine websterite-bearing alkali basalts of the Western Plateau and Danakil block¹⁰⁷.

The highest MgO contents yet measured in aphyric Ethiopian basalts lie in the range 10–12%^{11,14,80}, all from basalts in the Tertiary plateau pile. Lavas rich in olivine phenocrysts, with total MgO > 20%, form localized fissure flows on the rift valley floor, and also occur among the more alkaline of the plateau

basalts. Picritic rocks are a particular feature of the Ashanghi sequence: concentration of picritic lavas at low stratigraphical levels has been recorded from some other flood basalt provinces¹⁰⁸. A corollary of the picrite-parent model is that the crust is interleaved or underplated, depending on the type of magma chambers postulated, by dense ultramafic cumulates of similar volume to that of the erupted lava pile¹⁰⁶. For a large volcanic province such as the Ethiopian one, the corresponding cumulate volume implies that seismic profiling might well detect its presence, either at the base of the crust or contributing substantially to the lower part of a thickened crust²⁹.

A typical primary magma, generated in mid-Tertiary time beneath the Ethiopian plateaus-to-be, is considered by Piccirillo *et al.*⁶⁰ to contain 10.7% MgO and have an atomic Mg/Mg + Fe ratio of 0.66. Fractionation of 14% Fo₈₆ olivine, 6–12% An₈₀ plagioclase, and 8% clinopyroxene (Ca content decreasing with decreasing alkalinity of the magma) would generate the observed flood basalt geochemistry from this postulated primary magma. Such a fractionation scheme requires original Ni and Cr contents of 400 p.p.m. in the primary magma. The results of studies on other basaltic provinces of the world^{93,106,109}, however, indicate that the MgO, Ni and Cr values calculated for Ethiopia are distinctly low, raising afresh the possibility of either polybaric or open magma chamber processes¹¹⁰ controlling basalt evolution.

An estimate of pristine mantle compositions beneath Ethiopia, utilizing both analyses of xenoliths in alkali basalts¹¹¹ and incompatible trace-element data from the flood basalts⁶⁰, in turn enables an estimation of the degrees of partial melting required to produce these basalts. The results are as follows⁶⁰:

	% Melting range	Mean value
Termaber basalts	1–10	7
Alaji basalts	18–30	22
Aiba basalts	17–40	25
Ashanghi basalts	13–18	14

The highest degree of melting is seen to apply to the voluminous tholeiitic flood basalts of the Aiba Formation, the lowest to the alkaline central basalts of the Termaber Formation.

A surprising result stems from detailed geochemical comparison of Tertiary plateau with Quaternary Afar tholeiitic basalts (transitional basalts, in the tripartite classification): no essential distinctions can be made between the two suites. This is true for major elements, trace elements, and rare-earth element profiles^{60,102}. The only marginally significant differences are slightly higher SiO₂ and K₂O in the plateau suite¹¹², but these may merely express the secondary alteration of many of these older rocks. The Afar axial range basalts commonly show a greater iron-enrichment than occurs in most plateau basalts, suggesting a lower partial-pressure of oxygen in the present mantle beneath Afar²⁸. Otherwise the indistinguishable chemistry of the two suites underlines a similarity in mantle sources and melting processes, regardless of the structural setting expressed in crustal thickness and presumed geothermal gradient.

Recent geochemical studies on Ethiopian basalts have revealed what is, at first sight, a contradiction. An inverse relationship exists between basalt alkalinity and incompatible trace-element concentrations (rare-earths included), for example, for the basalts around the northern end of the rift valley. There, late Tertiary tholeiites of the plateau margins have higher incompatible trace-element contents than do superimposed mildly alkaline basalts of the rift floor^{92,112}. Again, in the interior of the western plateau, localized fissure alkaline basalts of Quaternary age^{11,43,61} are considerably depleted in incompatible and rare-earth elements compared with the underlying Tertiary tholeiites^{60,102}. Rather than posit some form of mantle metasomatism¹¹³ that was somehow restricted to tholeiite magma evolution, the authors concerned essentially concur that successive massive melting episodes, imposed on a closed mantle reservoir under the Ethiopian region, resulted in a progressive depletion of incompatible trace elements. Thus

the later the basalts generated, the more depleted their character regardless of their degrees of alkalinity¹¹² (basalt alkalinity is largely dependent on Na₂O content; sodium, of course, is not an incompatible element). Piccirillo *et al.*⁶⁰ further suggest that since the strongly depleted Quaternary basalts on the plateau occur some 250 km west of the mid-Tertiary fissure-swarm zone of the Afar margin, there may have been horizontal displacement of lithosphere by this distance relative to asthenosphere since Miocene time. However, Tertiary dykes are exposed south along strike from this plateau subprovince^{23,61}.

Strontium isotope data for Afar Quaternary basalts¹¹⁴ provide for a completely different mantle model from that discussed above. Emphasis in this alternative model is placed on a stack of compositional zones comprising the upper mantle beneath Afar. Continuing lithospheric extension permits the passive rise of progressively deeper, more depleted asthenosphere into the Afar melting zone¹¹⁴. By contrast with the previous model, the depletion is not the result of Cenozoic basalt generation but occurred at some unspecified earlier period in mantle evolution. A decision between these two alternatives may stem from extending detailed isotope studies to the tholeiitic and alkaline basalts of the plateaus, unless there is gross lateral heterogeneity in the Ethiopian mantle. However, the precisely similar ranges of ⁸⁷Sr/⁸⁶Sr values for flood basalts of plateaus and Afar^{114,115}, 0.7035–0.7040, argues against such heterogeneity. Barberi and co-workers^{27,28,102} have strongly criticized the concept of an enriched deep-mantle plume¹¹⁶ actively impinging on the base of the lithosphere under Ethiopia.

Geodynamic considerations and conclusions

The two most voluminous basaltic outpourings in the Ethiopian–Yemen region occurred during the intervals 30–

15 Myr and 4.5–0 Myr. The most intense activity was concentrated in the earlier part of each of these intervals^{29,39,117,118}. The intervals coincide with those proposed by Girdler and co-workers^{119,120} for a two-stage opening of the Red Sea. The intervening, 15–11 Myr rhyolitic episode could then be seen as a thermal response to cessation of continental lithospheric attenuation. Basaltic injection into the lower crust would have lain stagnant, facilitating either anatexis of sialic rocks or large-scale fractionation²⁹.

The two major basaltic episodes in Ethiopia were (and are) coeval with the development of continental margins at the junction of the Red Sea and Gulf of Aden. Throughout the Neogene and Quaternary, volcanism has been focused on this junction within some 500 km from the triple-point^{15,23}, in such a way as to suggest the existence of an underlying deep-mantle plume, whatever its chemical characteristics. The mid-Tertiary flood basalts, excluding early localized eruptions of weakly alkaline basalts, show a clear evolution from voluminous oversaturated andesine tholeiites through increasingly restricted and more alkaline types (Table 2). This evolution is consistent with declining rates of lithospheric attenuation and progressively more restricted and/or deeper mantle melting^{60,92}.

The message of this review, which would be reinforced if silicic volcanics were to be included, is surely that Ethiopian volcanological studies remain in their infancy, for all the stimulating progress of the past decade. The next crucial step must be an unravelling of the plateau basalt stratigraphy and location of the feeder-fissures. Geochemical and isotopic studies can then follow, leading to an integrated understanding of magmatic and tectonic episodicity in a remarkable flood basalt province whose evolution is still proceeding. Blanford's call for attention echoes after more than a century.

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ARTICLES

Are QSOs activated by interactions between galaxies?

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In a sample of 45 quasistellar objects (QSOs) with redshift 0.62 or less, a large fraction (>30%) appear to be currently interacting with another galaxy. All but three of the QSOs reside within nebulosity interpreted as a surrounding galaxy, and about one third have spiral galaxy characteristics. About one third appear to reside in clusters or small groups of galaxies. The present observations support the view that the QSO phenomenon is fundamentally linked to interactions between galaxies in clusters or smaller groups.

THE principal morphological findings in a programme of high-resolution imaging of QSOs carried out with the Canada-France-Hawaii Telescope are summarized in the article. The data consist of direct or image tube enhanced photographs in *B* and *R* passbands. The instrumental resolution is 0.25 arc s, and the image quality (seeing) was 0.6-1.0 arc s (FWHM) in almost all cases. Limiting brightnesses are ~24 mag per arc s² in *R* and ~25 mag per arc s² in *B*, over radii of ~10 arc s. *R* band maps and photometric quantities have been published elsewhere^{1,2}, where we also discussed the evidence which suggests that the QSOs are active nuclei of galaxies of normal luminosities and sizes. In more recent work, we have obtained data in two colours to study the stellar populations and galaxy types. Selection criteria for the sample have been to observe objects distributed approximately evenly in redshift, luminosity and discovery type (radio, optical and X-ray). The sample was not chosen by any previous knowledge or prejudice as to the individual morphologies and, in fact, at the lowest redshifts ($z < 0.1$), we have observed about 30% of all known objects. Figure 1 shows the redshift and luminosity distribution and Table 1 summarizes the principal sample characteristics and morphological properties. (Table 2 itemizes the objects and the principal characteristics discussed below.)

The present results are from a larger data base than the earlier publications. We intend to publish elsewhere full details of all objects studied, including underlying galaxy maps, characteristics and luminosities, for a still larger sample of data. There are, however, three points we wish to make here from these studies. The principal one is that a large fraction of the sample of QSOs appears to be currently interacting with another galaxy. There are six cases in which there is a definite connecting link

of luminous material between nuclei, or extra luminosity surrounding two symmetrical nuclei. In four of these cases the QSO and galaxy redshifts are the same to within a few hundred km s⁻¹, and in the remaining two the extra nucleus lies well within the QSO galaxy. There are nine further cases in which nearby galaxies are connected by a luminous bridge to the QSO in our data, but we lack the redshift confirmation, at present. However, these are not simply projection effects on the sky—the connecting luminosity lies well beyond the lowest isophotes of the objects in other directions. Some of these objects have been discussed in detail elsewhere^{3,4}. We give below brief details

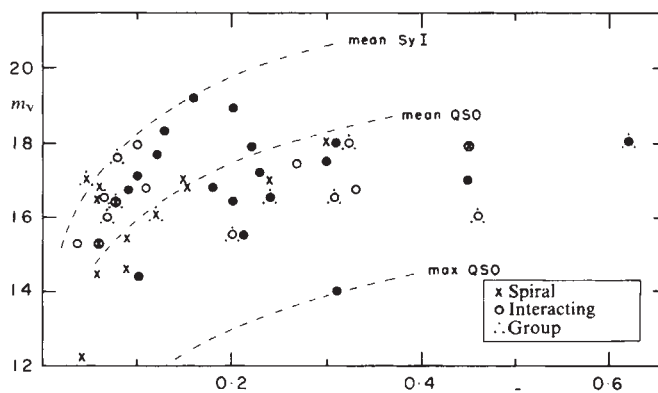


Fig. 1 Distribution of sample QSOs in magnitude and redshift. Dashed lines are constant luminosity ($q_0 = 0$) loci for (top to bottom) mean of ~30 bright Seyfert 1 galaxies; mean of Hewitt and Burbidge¹³ catalogue QSOs in redshift range 0.1-0.3; high luminosity QSO envelope (3C273).

Table 1 QSO galaxy statistics

	Whole sample no. (%)	Discovery type			Redshift	
		X-ray	Radio	Optical	<0.1	>0.1
Total	45 (100)	19	15	11	17	28
Interacting	15 probable (33) 6 definite (13)	7	5	3	7	8
Spiral/bar	15 (33)	7	3	5	9	6
Group present	13 (29)	6	6	1	6	7
Irregular	2	1	1	0	1	1
Elliptical	1	1	0	0	1	0

on some of the newer discoveries. Overall, we consider that some 30% of our sample is in a current state of interaction, as defined above. This fraction is essentially the same for all three discovery types of QSO, and for high and low redshifts in our sample (Table 1).

The second point is that a large proportion of the objects appear to have companion galaxies, in addition to those we regard as interacting ones. Our evidence here is of two kinds. In some cases the QSO lies in a group of galaxies whose brightness, size, luminosity profile and scale height, and spatial distribution suggest association with the QSO galaxy, assuming the QSO redshift applies to all. Furthermore, these objects have a significantly higher population in the sky close to the

QSO than several arc minutes away. In other cases there are only a few (2–4) neighbouring objects, but there are published spectroscopic redshifts which show them to be the same as the QSO. In a few cases, both kinds of evidence exist. In some cases, other published work has suggested the same conclusion^{5–7}. However, we have not counted any cluster or group that we have not detected (in most cases independently) in our own data. Thus, some 30% of our sample have companion galaxies. The proportion holds for all subgroups (Table 1) with the possible exception of the optically selected sample. (One can imagine reasons for this, if true, but we prefer to wait and see if the result holds in a larger sample.) Nine of the 15 interacting QSOs lie in groups or clusters. We do not regard that the remaining six fields have yet been researched exhaustively for companions.

The third principal point concerns the morphological type of the underlying QSO galaxies. In 15 cases we see definite characteristics of spiral type galaxies: 10 of these are bars, seen either as bars with trailing blue arms, or a 90° rotation between inner and outer isophotes with large ellipticity. In three cases we see an edge-on galaxy with central bulge, and in two we see blue arms as in a face-on spiral. In a further 12 cases we do not have sufficient information to make any morphological statement, but in most (~25) of the objects in the whole sample,

0906 + 484

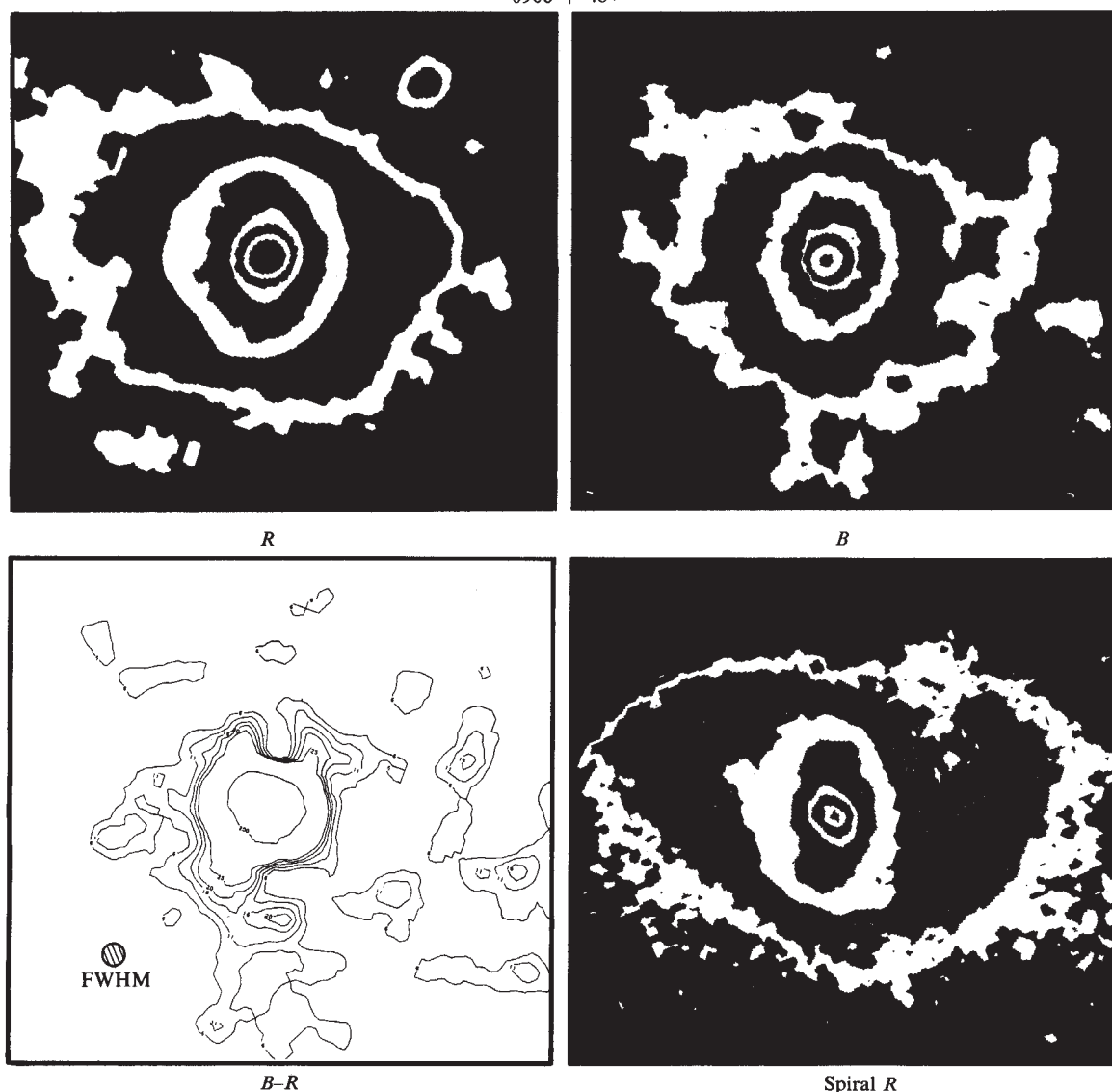


Fig. 2 Top: QSO images in *R* and *B* light. Pictures cover ~30 arcs in sky. North is up and east to the left. Contour levels separated by $2 \times$ intensity. Note 90° isophote rotation between inner and outer parts, characteristic of barred spirals. *R* image shows two faint galaxies to the west and north-west of the QSO. Bottom: *B-R* intensity difference shows blue inner arms of QSO. Barred spiral 3 arc min away in sky shown on same scale, is ~1 mag brighter than QSO galaxy, but very similar in morphology.

the isophotes are very irregular, having no rotational or mirror symmetry. In many cases the luminosity profile is best fit with an exponential law outside the point spread function, and in only one is it clearly fit with a $r^{-1/4}$ law. In many cases, however, deconvolution of the point spread function of the QSO nucleus makes model fitting to the luminosity profile ambiguous between an exponential and an $r^{-1/4}$ law. Only two of the currently interacting QSOs have barred structure.

Before discussing the significance of these three points we wish to present some details on the better examples of these. Two interacting systems have been presented elsewhere³: 0351+026 ($z=0.036$, X-ray selected) and 1229+204 ($z=0.065$, optically selected^d).

0906+484 and 0225+312

0906+484 is an optically selected object at $z=0.12$, it has a strong bar and a 90° rotation of isophotes outside of it (Fig. 2). The $B-R$ picture shows a blue arm which starts near one end

of the bar and a fainter arm may be seen on the opposite side. Another barred galaxy lies 3 arc min away to the south-east which is very similar in size and morphology (Fig. 2), but whose extended luminosity is about 1 mag brighter than the QSO galaxy. Another object with a very clear two-armed barred spiral structure is 0226+312 (Fig. 4), an X-ray selected QSO at $z=0.06$.

1747+684

This object was identified from X-ray data by Kriss and Canizares⁸, as an interacting pair at the same redshift. Our data (Fig. 3) show that the northern object has the colour, shape and luminosity of an elliptical galaxy. The QSO is 0.5 m bluer in $B-V$, with similarly blue fuzz. A hulge of blue luminosity (bluer than the QSO, as in 1229+204) is found in the southern part of the normal galaxy, suggesting the presence of hot young stars. A third component, also nonstellar, lies to the south, somewhat redder than the normal galaxy, much smaller and

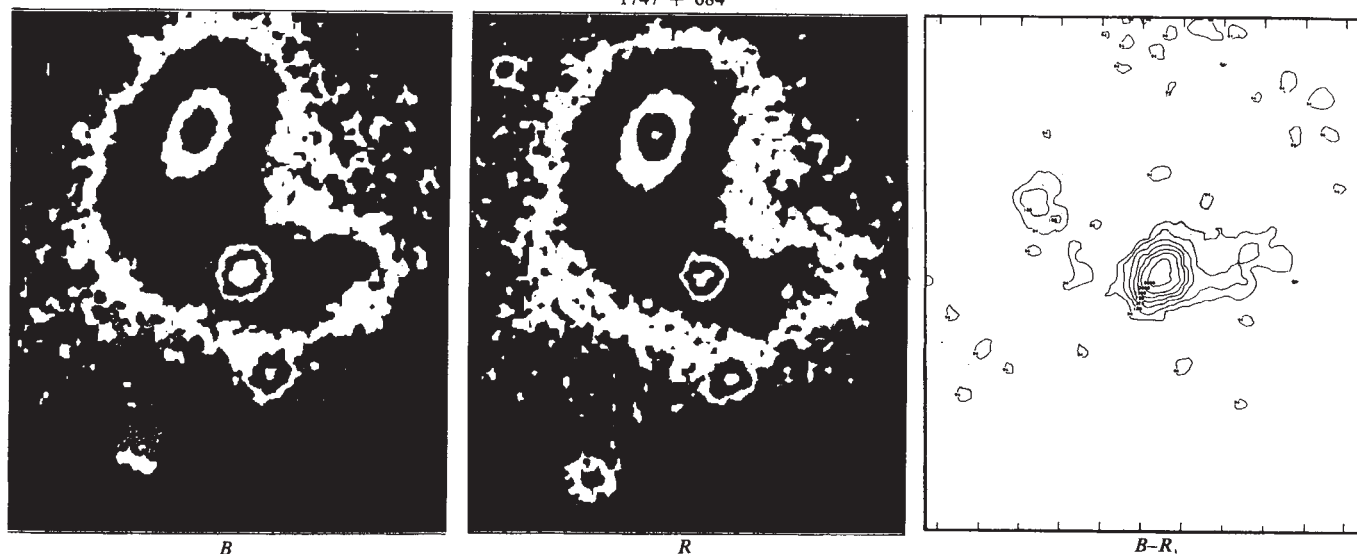


Fig. 3 Colour images of X-ray selected object 1747+684. QSO is at picture centre. Other bodies are nonstellar. Main galaxy has shape and colour of elliptical galaxy and has redshift close to that of QSO. $B-R$ intensity difference normalized to elliptical galaxy nucleus shows significant extended blue light near QSO and in south-east bulge of elliptical galaxy. $B-V$ is 0.3 to 0.6 in these regions. Picture details such as orientation, scale, resolution and contour interval as for Fig. 2.

Table 2 List of objects and some general properties

Object	m_v	z	Discovery type*	Morphology†	Object	m_v	z	Discovery type*	Morphology†
0007+106	15.4	0.09	O	S	0957+227	17.9	(0.1?)	R	(I)
0037+061	16.5	0.06	X	S	1020-103	16.4	0.20	R	
0132+077	17.0	0.15	R	S	1059+730	14.7	0.09	X	S C
0134+033	17.6	0.08	O	C (I)	1225+085	16.7	0.09	X	
0147+089	17.4	0.27	O	(I)	1226+1336	16.9	0.15	X	S
0148+090	17.5	0.30	O		1227+1403	17.1	0.10	X	
0214-033	16.8	0.33	X	(I)	1229+204	15.3	0.06		S I
0225+312	16.8	0.06	X	S	1248+337	18.9	0.19	O	
0241+622	16.4	0.04	X	S	1400+162	16.5	0.24	R	C
0242-387	17.7	0.12	O		1525+1551	17.2	0.23	X	
0351+026	15.3	0.04	X	I	1747+684	16.5	0.06	X	C I
0357+104	16.8	0.18	X		2130+099	14.5	0.06	O	S
0607-157	18.0	0.32	R	C (I)	2135+147	15.5	0.20	R	C I
0721+69	16.8	0.11	X	(I)	2141+175	15.5	0.21	R	
0742+318	16.0	0.46	R	C (I)	2205-167	19.2	0.16	O	
0752+258	17.0	0.45	R		2217+08N	18	0.62	R	C
0754+393	14.4	0.10	X		2217+08S	18	0.23	R	
0840+503	18.1	0.30	O	S	2247+140	16.9	0.24	R	S
0844+377	17.9	0.45	X	S C (I)	2251-1751	16.5	0.07	X	S C I
0845+378	18.0	0.31	X		2251-178	16.0	0.07	X	C I
0851+202	14.0	(0.31)	R		2305+187	16.5	0.31	R	C (I)
0906-091	18.3	0.13	X		2331-240	17.0	0.05	R	S C
0906+484	16.1	0.12	O	S					

* X, X-ray; O, optical; R, radio.

† S, spiral characteristics; C, cluster or group; I, interacting.

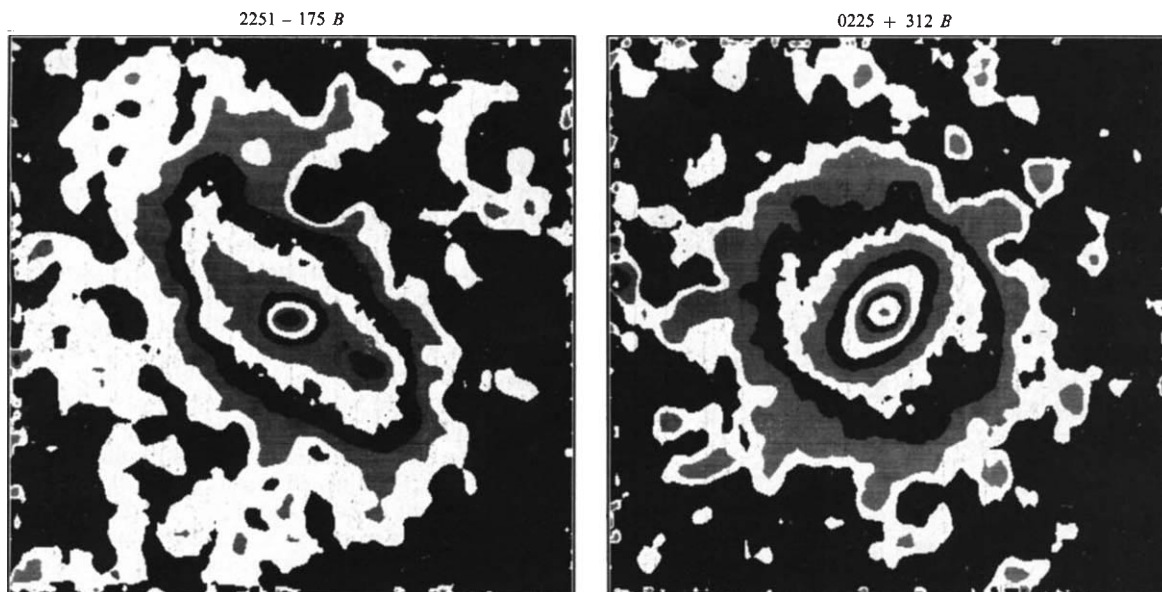


Fig. 4 Blue images of two QSOs. 2251-175 has barred structure with luminous blue knot in western arm ($B-V=0.4$, $M_V=-16.5$). Knot lies due west of nucleus. 0225+312 has obvious two-armed barred spiral structure. Other picture details as for Fig. 2.

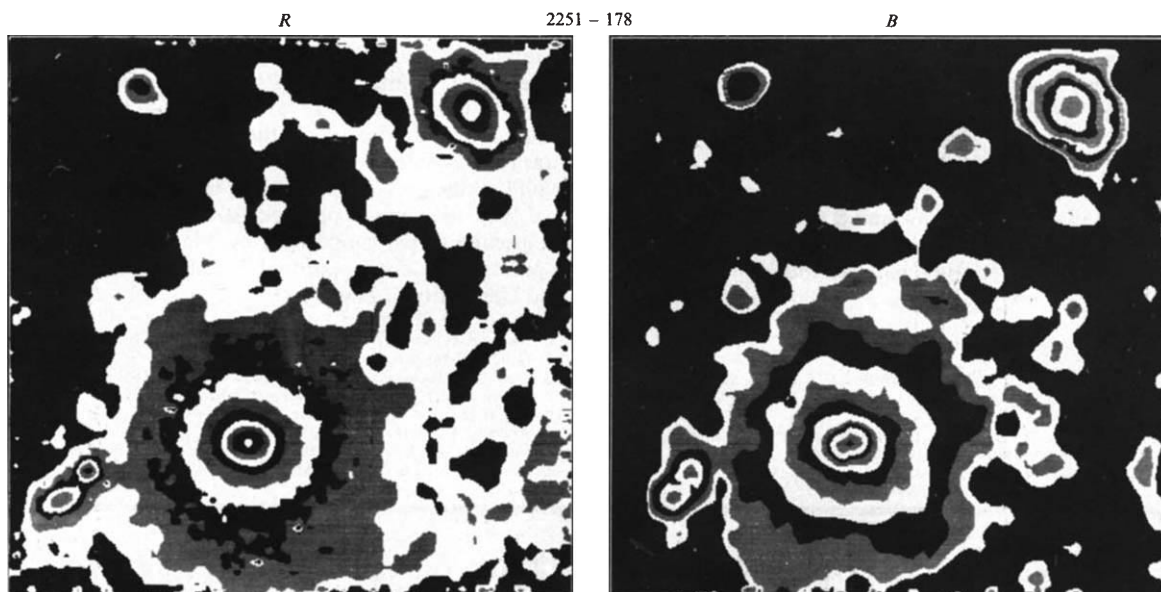


Fig. 5 R and B images of 2251-178. QSO is luminous extended object in lower part. Upper body is radio galaxy due north of QSO. Note connecting R luminosity and B luminosity extended in this direction. Objects to south-east of QSO are a star and a faint galaxy. Picture side 56 arcs, contour intervals $2\times$.

2.5 mag fainter. The QSO fuzz is swept back on both sides, reminiscent of a tailed radio galaxy.

2251-1751

This and 2251-178 lie in the same cluster^{7,8}; both were discovered as X-ray sources. The underlying galaxy has a barred structure (Fig. 4) which contains a bright, very blue knot in it, and whose ends curve away like the inner arms of a barred spiral. The eastern arm is brighter and could conceivably be a tidal tail caused by interaction with the blue object in the western arm. Another galaxy lies 30 arcs to the north, also near enough to be connected with the eastern arm.

2251-178

This QSO has an emission line radio galaxy 40 arcs to its north (Fig. 5). The QSO galaxy is irregular, but extended towards the companion galaxy. In red light ($H\alpha$ lies in the centre of our R band) there is extended luminosity connecting the north-west side of the QSO with the companion. The emission line

galaxy and the QSO galaxy are both bluer than the central galaxy of the cluster. A small red galaxy lies 20 arcs to the south-east of the QSO, but does not appear to be connected. The emission line galaxy to the north has two nuclei, or a dust lane through the nucleus.

2305+187

This is the radio quasar 4C18.68, whose radio structure has been modelled as a precessing jet by Gower *et al.*⁹. Our imaging (Fig. 6) does not indicate radio-associated optical luminosity, but does show the object to lie in a small group of galaxies, whose closest neighbours are shown in Fig. 6. The QSO morphology and colours resemble an edge-on spiral. The red object to the north-east is nonstellar and is placed away from an extension of luminosity from the QSO. We do not claim optical evidence for an interaction here (although the north-east extension of the QSO galaxy is suggestive). However, the precessing radio jet model suggests a continuing close interaction near the galactic nucleus. The optical data suggest a compact

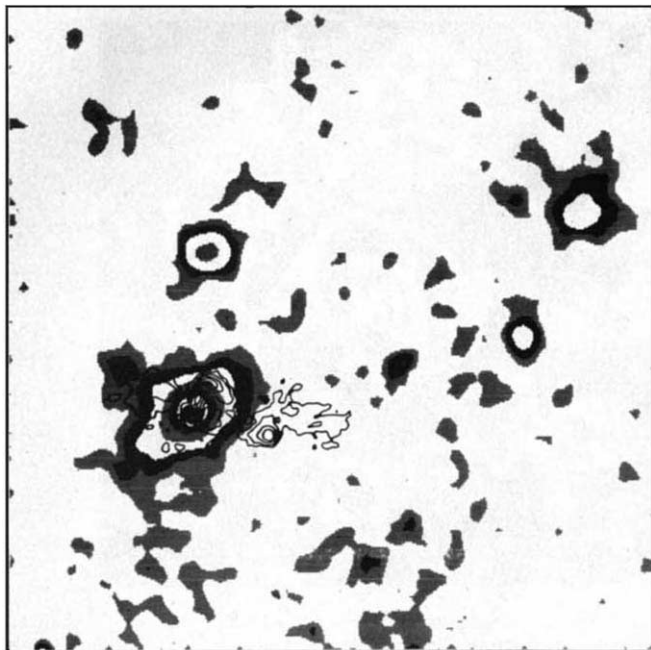


Fig. 6 *R* image of 4C18.68 = 2305 + 187. All objects are non-stellar. QSO (largest object) has elongated image with faintly luminous body to the north-east. The QSO host is bluer than any other body in field. 6-cm radio contours are overlaid. These data have been fitted with precessing jet model⁹. Picture details as for Fig. 2.

group in which interactions are likely to occur. Yee and Green¹⁰ find the 70 arc s diameter field around 2305 + 187 to contain seven times the expected chance number of galaxies to our limit of detection. The foreground galaxies found by Stockton¹¹ lie outside the area shown in Fig. 6.

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Conclusions

Clearly, in all the examples discussed here (and many others), detailed spectroscopic investigation and higher resolution imaging (with the Space Telescope) are needed to tell us much more about the details of each situation. Nevertheless, there are some very strong indications in the results so far. The very high proportion of current interactions, group membership and barred characteristics suggest that the QSO phenomenon may be the activation of a galactic nucleus by tidally accreted gas from an encounter with another galaxy. In our data, the QSO lies in a large galaxy of spiral characteristics, with neighbours which are generally smaller and less luminous. Interactions which power a QSO would need to feed gas to the active nucleus, so that either gas must be stripped from a gas-rich neighbour, or it must exist in the QSO galaxy and be perturbed into the nucleus. Thus, smaller neighbours would be stripped, or gas-poor neighbours would act as perturbing forces; either way only one QSO would result. Cases where both nuclei are activated might be rare, and the object 2135-147 is perhaps the only known example of this⁵. However, the existence of gas is not sufficient: it must be funnelled to the nucleus, and it seems significant that barred structure is regarded by some¹² as a way of doing this. M. De Robertis (personal communication) argues that all QSOs may be activated in this way, and that present epoch activity occurs in small groups or clusters where stripping and virialization have occurred more slowly than in large clusters. The lifetime considerations of visible interactions and individual QSO activity make the fraction of cases in which interaction is seen, compatible with expectation. Finally, the presence of areas of blue luminosity and more extended red luminosity in these objects, is suggestive of young star formation and neutral hydrogen (H α) emission which may well be other products of the events igniting the QSO.

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Ca-Al-rich chondrules and inclusions in ordinary chondrites

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Ca-Al-rich objects, hitherto mostly found in carbonaceous chondrites, are shown to be widespread, albeit rare, constituents of type 3 ordinary chondrites. Widespread occurrence and textural similarities of Ca-Al-rich chondrules to common, Mg-Fe-rich chondrules suggest that they formed by related processes. It is suggested in this article that Ca-Al-rich chondrules were formed by total melting and crystallization of heterogeneous, submillimetre- to millimetre-sized dustballs made up of mixtures of high-temperature, Ca-Al-rich and lower-temperature, Na-K-rich components.

SINCE their discovery in the CV chondrites Leoville¹, Lance² and, particularly, Allende³, Ca-Al-rich inclusions (CAIs) in carbonaceous chondrites have become the objects of intensive study. This interest was sparked by the discovery in the inclusions of abundant refractory minerals rich in Ca and Al, suggesting that they probably formed at high temperatures during the early history of the Solar System⁴. Various types of

inclusions that differ in texture, shape, grain size and bulk and mineral compositions have been described and several schemes for their classification have been proposed elsewhere⁵⁻⁹.

Ca-Al-rich objects, although abundant in carbonaceous chondrites, have only been observed in a few type 3 and 4 ordinary chondrites¹⁰⁻¹⁴. Noonan¹⁰ investigated 80 type 3 and 4 ordinary chondrites and found Ca-Al-rich objects in only

five. He reports Ca-Al-rich chondrules as well as irregularly-shaped inclusions with high Na₂O-contents. Both types contain mineral assemblages similar to those described here. He concludes that Fe-spinel and nepheline within these objects may be primary phases and that Na and Fe are not secondary and did not enter the Ca-Al-rich objects by diffusion from the host chondrite matrix. Furthermore, Fudali and Noonan¹¹ describe a rutile-spinel-ilmenite chondrule from the Gobabeb, H4 chondrite and Noonan and Nelen¹² report a black fragment containing the minerals fassaite, forsterite and Fe-spinel, as well as an irregularly-shaped inclusion enriched in Ti, Al, Ca and Na in the Weston chondrite regolith breccia. Nagahara and Kushiro¹³ were the first to publish bulk compositions of Ca-Al-rich chondrules and demonstrate the chemical similarities of those objects in carbonaceous and ordinary chondrites. They suggest that Ca-Al-rich chondrules could represent primarily early condensates that were melted and cooled at a rate similar to that of Mg-Fe-rich chondrules. Recently, Wlotzka¹⁴ described Ca-Al-rich chondrules in the Tieschitz type 3 ordinary chondrite.

Dodd¹⁵ suggested that carbonaceous and ordinary chondrites have different origins. This was mainly based on the fact that Ca-Al-rich objects are abundant in carbonaceous but rare in ordinary chondrites. Similarly, McSween¹⁶ concluded that every chondrite group should have its own distinctive population of inclusions and/or chondrules. However, Scott *et al.*¹⁷ argued that carbonaceous and ordinary chondrites share common origins and that the chondrules and CAIs formed by related processes very early in the history of the solar nebula. In the present study we find that all type 3 ordinary chondrites we investigated contain Ca-Al-rich objects that are quite similar in bulk and mineral compositions to coarse-grained CAIs¹⁶ and fine-grained (type F) aggregates⁸ described from the Allende carbonaceous chondrite. Some of the objects from ordinary chondrites are round and clearly formed from a melt, as did the common, abundant Mg-Fe-rich chondrules and are therefore, justifiably referred to as Ca-Al-rich chondrules. We



Fig. 1 Ca-Al-rich chondrule in the Dimmitt chondrite regolith breccia (~400 μ m in apparent diameter) (Table 1, no. 1). Fassaite crystallized radially from the rim into the core of the chondrule. Euhedral spinel grain is indicated by the arrow.

Table 1 Bulk compositions (in wt %) of Ca-Al-rich objects in ordinary chondrites

Object no.	Meteorite	SiO ₂	TiO ₂	Al ₂ O ₃	Cr ₂ O ₃	FeO	MnO	MgO	CaO	Na ₂ O	K ₂ O	Total	Observed phases
1	Dimmitt	41.4	1.09	22.2	0.13	4.7	<0.02	8.6	19.6	0.49	0.04	98.25	F, S, Pl, CP, O, M _c
2	Dimmitt	45.3	0.97	19.5	0.18	4.1	<0.02	15.0	9.8	3.5	0.34	98.69	F, M _n
3	Sharps*	49.4	0.80	18.0	0.33	1.8	NA	14.5	11.9	2.3	0.07	99.1	F, O, Me, M _c
4	Sharps	43.2	0.55	15.6	0.14	9.0	NA	22.5	8.9	1.08	0.08	100.85	O, M _c
5	Sharps	51.5	0.18	21.5	0.19	9.3	NA	3.8	10.5	3.6	0.08	100.65	Pl, M _{cn}
6	Sharps	42.9	0.53	13.0	0.13	4.9	NA	25.4	12.3	0.46	0.11	99.73	F, O, M _c
7	Sharps	52.0	0.95	20.7	0.20	0.76	NA	11.4	13.0	2.3	0.08	101.19	F, O, M _c
8	Quinyambie	54.3	0.66	19.1	0.72	1.5	0.22	8.5	12.7	4.0	0.36	102.04	P, Pl
9	Dhajala	44.5	0.53	15.4	0.14	8.1	NA	19.4	7.9	1.9	0.10	97.97	O, M _c
10	Tieschitz	45.5	0.38	11.0	0.50	3.7	NA	28.0	7.7	1.08	0.08	97.94	F, O, Pl, M _c
11	Piancaldoli	43.3	0.65	17.0	0.14	0.43	NA	28.4	10.2	0.34	<0.03	100.46	F, O, M _c
12	Bremervörde	49.3	0.77	19.4	0.17	2.7	NA	12.5	12.9	1.8	0.17	99.71	F, O, M _c
13	Bremervörde	48.5	0.49	12.2	0.47	2.3	NA	23.5	7.7	1.9	0.09	97.15	O, M _c
14	Bremervörde	42.8	0.47	16.2	0.36	7.1	NA	16.8	4.4	7.4	0.06	95.59	S, N, O, M _n
15	Ngawi	44.2	0.83	18.7	0.17	0.65	<0.02	23.6	12.3	0.89	0.05	101.24	F, O, M _c
16	ALHA78084	50.6	0.63	17.7	0.47	9.2	0.19	7.4	8.0	4.6	0.16	98.95	F, M _{cn}
17	ALHA78084	53.9	0.35	16.2	0.56	5.4	0.25	13.4	8.5	3.6	0.12	102.28	O, P, M _c
18	ALHA78084*	40.3	0.32	12.4	1.35	14.5	NA	25.6	4.4	1.5	0.06	100.43	S, O, Pl, Me
19	ALHL78084	53.9	0.67	17.3	0.22	4.6	0.17	11.2	10.0	3.9	0.18	102.14	O, Pl
20	Weston	47.0	0.78	20.3	0.07	2.0	NA	10.4	17.6	1.5	0.04	99.69	F, S, O, M _c
21	Willaroy	48.6	0.58	15.3	0.30	5.9	NA	18.4	9.8	1.6	0.08	100.54	O, M _c
Avg. chondrules		47.2	0.63	17.1	0.33	4.6	NA	16.6	9.7	2.4	0.11	97.97	
22	Sharps†	31.3	0.52	39.8	0.12	9.0	NA	5.7	5.4	7.5	0.10	99.44	H, N, S, M _n
23	Sharps	13.5	1.69	46.3	0.12	12.7	NA	13.0	5.7	2.6	0.41	96.02	F, S, CP, Pe, I, M _n
24	Weston	43.7	0.91	21.5	0.13	5.7	0.07	10.7	9.6	7.6	0.63	100.54	F, N, O, M _n
25	Weston	40.2	0.58	26.0	0.48	4.5	<0.01	8.8	11.5	6.4	0.51	98.97	F, S, CP, M _n
26	Weston	31.7	1.14	36.2	0.10	7.6	<0.05	7.6	8.2	5.9	0.53	98.97	F, CP, M _{cn}
27	Dhajala	50.2	0.61	16.5	0.52	3.6	NA	13.9	11.9	1.8	0.13	99.16	M _c
Avg. inclusions		35.1	0.91	31.3	0.25	6.0	NA	10.0	8.7	5.3	0.39	97.95	
Avg. (type F) aggregates ⁸		32.3	0.75	31.5	0.19	6.0	NA	11.3	12.2	4.5	0.29	99.49	
Avg. coarse-grained CAIs ¹⁹		32.9	0.74	26.30	0.19	9.31	0.09	11.82	11.27	4.33	0.30	97.25	

Objects 1-21: chondrules. Objects 22-27: irregularly-shaped inclusions.

* Bulk compositions of nos 3 and 18 without metal grains; NA, not analysed. Observed phases: S, spinel; F, fassaite; O, olivine; Pl, plagioclase; P, low Ca-pyroxene; CP, high Ca-pyroxene; H, hibonite; N, nepheline; Pe, perovskite; I, ilmenite; Me, metal; M_c, Ca-Al-rich, fine-grained mesostasis, CaO > 7.5 < 20.0 wt %; M_n, Na-Al-rich, fine-grained mesostasis, Na₂O > 4.5 < 16.1 wt %.

† Bulk composition of the entire chondrule-like object.

predict that Ca–Al-rich chondrules and inclusions are ubiquitous, albeit rare, constituents of type 3 ordinary chondrites. We conclude that their occurrence in both carbonaceous and ordinary chondrites provides additional evidence for the suggestion by Scott *et al.*¹⁷ that carbonaceous and ordinary chondrites and their constituents formed by related processes.

Analytical techniques

We studied using the optical microscope in transmitted and reflected light 11 polished thin sections of 8 type 3 ordinary chondrites, Bremervörde (H; UNM=University of New Mexico 579), Dhajala (H; UNM 127), Ngawi (LL; USNM=National Museum of Natural History, Washington DC 2483-2), Piancaldoli (LL; USNM 5649), Quinyambie (L or LL; USNM 6076), Sharps (H; USNM 640-1), Tieschitz (H; UNM 307), and Willaroy (H; USNM 5708-1), one H4 ordinary chondrite, Allan Hills A78084, 135, and two chondrite regolith breccias containing unequilibrated material, Dimmitt (H; UNM 532) and Weston (H; USNM 1180-1). Bulk and mineral compositions of Ca–Al-rich chondrules and inclusions were determined with an ARL EMX-SM electron microprobe operated at 15 keV accelerating potential and about 20 nA sample current. Bulk compositions were determined with a broad (40–80 μm) electron beam. Bulk and mineral analyses were corrected by standard procedures¹⁸.

Results

We found 27 Ca–Al-rich objects in the 11 polished thin sections studied. Based on their shape, texture and bulk composition they can be divided into two groups, Ca–Al-rich chondrules and Ca–Al-rich inclusions.

Ca–Al-rich chondrules: Ca–Al-rich chondrules are well-defined, round objects, varying in apparent diameter from 0.1 to 0.8 mm (Fig. 1). They are much smaller than most Ca–Al-rich objects in Allende. All have igneous textures and clearly crystallized as individual, molten droplets, just like the common Mg–Fe-rich chondrules. These Ca–Al-rich objects can, therefore, be termed chondrules. They were found in the H-group chondrites Dimmitt, Sharps, Dhajala, Tieschitz, Bremervörde, Allan Hills A78084, Weston and Willaroy, in the L- or LL-group chondrite Quinyambie, and in the LL-group chondrites Piancaldoli and Ngawi (Table 1). They are the most commonly observed Ca–Al-rich objects in ordinary chondrites, amounting to 21 out of 27 occurrences.

Ca–Al-rich chondrules commonly consist of elongated, partly skeletal crystals, mainly of Al–Ti-rich pyroxene (fassaite), as well as olivine, embedded in a fine-grained, microcrystalline to glassy matrix. In some cases, the matrix is stoichiometric CaO-rich plagioclase (An_{70–86}), in others it appears to be a fine-grained intergrowth of various crystalline phases or glass. In fact, some chondrules are so fine-grained that quantitative electron microprobe analysis of individual mineral grains was not possible. Fassaite compositions vary widely, particularly in CaO (12.0–25.2 wt %), MgO (6.3–23.1), and Al₂O₃ (9.8–24.2)

(see also Table 2). Olivine is mostly MgO-rich (Fo_{95.0–99.8}), although in some chondrules, olivine of Fo_{73.7–87.1} was found. Euhedral spinels in chondrules are hercynian, with 14.4–17.4 wt % MgO, 14.8–17.6 FeO and 63.8–66.9 Al₂O₃. In one chondrule (no. 8, Tables 1 and 2), hercynian-chromian spinel grains with 11.3–13.1 wt % Cr₂O₃ and 54.7–55.1 Al₂O₃ were found. Accessory minerals in Ca–Al-rich chondrules are high- and low-Ca pyroxene and nepheline. Ca–Al-rich chondrules are generally devoid of metallic Fe, Ni; two of them (nos 3, 18, Table 1) contain one ~30 μm Fe, Ni grain each.

Ca–Al-rich inclusions: Ca–Al-rich inclusions are irregularly-shaped, roughly 100–250 μm in apparent diameter, and quite similar to spinel-rich, complex CAIs⁹ and fine-grained (type F) aggregates⁸ described from the Allende carbonaceous chondrite. We found these inclusions in the H3 chondrites Sharps and Dhajala and the H group regolith breccia Weston. They are usually layered in appearance with onion-shell-like rims, and are fine-grained. The rims consist mainly of fassaite and/or Ca-rich pyroxene. Constituent mineral grains are usually $\leq 10 \mu\text{m}$ in size and include spinel, hibonite, perovskite, ilmenite, fassaite, Ca-rich pyroxene, accessory olivine (mainly Fo_{96–99}) and a fine-grained mesostasis generally higher in Na₂O (5.0–16.1 wt %) than that of the Ca–Al-rich chondrules. MgO and FeO contents of spinel in Ca–Al-rich inclusions vary between 12.6 and 20.6 wt % and 8.2 and 19.1 wt %, respectively. Fassaite is relatively low in Al₂O₃ (8.0–12.0 wt %) and relatively high in CaO (21.4–24.6 wt %) (see Table 2).

One inclusion (no. 23, Table 1) contains rounded to irregularly-shaped objects ~25 μm in size that consist almost entirely of spinel, and are embedded in a fine-grained, Fe-, Na- and Cl-rich mesostasis (Fig. 2). The mesostasis material which consists partly of nepheline seems to be of secondary origin, that is, it was introduced after condensation of the object but before accretion of the meteorite. MgO in the spinel was substituted by FeO; small grains of perovskite in the spinel indicate substitution of CaO by FeO. One grain was found with a core of perovskite and a rim of what seems to be ilmenite, whereas others were completely transformed to ilmenite. The entire inclusion is surrounded by a shell of Ca-rich pyroxene and sometimes fassaite.



Fig. 2 Backscattered electron image of part of a 250- μm Ca–Al-rich inclusion in the Sharps chondrite (Table 1, no. 23). It consists of an onion-shell-like rim of fassaite and Ca-rich pyroxene (f), and rounded to irregularly-shaped objects that consist almost entirely of spinel (s) embedded in a fine-grained mesostasis (m). Perovskite (p) is partly transformed to what appears to be ilmenite (i).

Table 2 Selected analyses (in wt %) of minerals in Ca–Al-rich chondrules and inclusions

	Spinel		Hibonite		Fassaite	
	1	18	25	22	1	24
SiO ₂	NA	NA	NA	NA	40.8–43.5	47.9–49.6
TiO ₂	0.14	0.07	0.19	1.8	1.0–2.5	3.1–3.6
Al ₂ O ₃	66.8	55.1	64.0	87.4	16.9–24.2	9.6–11.9
Cr ₂ O ₃	0.25	11.7	0.42	—	0.1–0.2	0.2–0.3
FeO	14.9	16.1	19.1	0.9	0.1–0.9	0.2–0.3
MnO	0.15	0.29	NA	NA	NA	NA
MgO	17.3	14.3	13.7	0.9	10.4–12.0	14.2–15.6
CaO	0.05	0.36	0.26	8.4	23.4–25.2	22.8–23.6
Na ₂ O	0.05	0.16	0.17	0.1	<0.06	<0.06
K ₂ O	NA	NA	NA	NA	<0.13	NA
Total	99.64	98.08	97.84	99.5	(98.0–102.0)	

NA, not analysed. 1, Dimmitt; 18, Allan Hills A78084; 24, 25, Weston; 22, Sharps. Spinels are higher in FeO and fassaite lower in TiO₂ than those in most Allende CAIs.

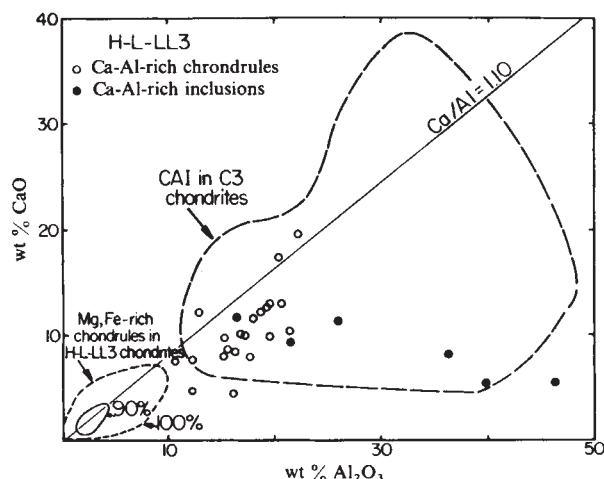


Fig. 3 CaO/Al₂O₃ variation of Ca-Al-rich objects in ordinary chondrites. The outlined field for CAIs in C3 chondrites is after McSween¹⁶. Data for Mg-Fe-rich chondrules are from refs 19–21.

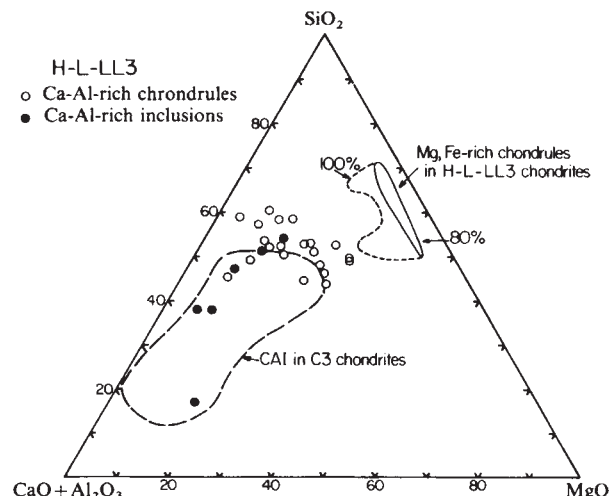


Fig. 4 Bulk compositions of Ca-Al-rich chondrules and inclusions in ordinary chondrites. SiO₂ tends to be higher in objects from ordinary chondrites. Same references as in Fig. 3.

Another irregularly-shaped inclusion (no. 22, Table 1) is incorporated in the core of a rounded, chondrule-like object. It consists of anhedral hibonite grains that are completely coated by spinel. The mesostasis around this hibonite-spinel assemblage is mainly nepheline with small insets of what appears to be sodalite. The chondrule-like object host is higher in Al₂O₃ and CaO than common Mg-Fe-rich chondrules and consists of high- and low-Ca pyroxene, olivine, CaO-rich plagioclase and small Fe, Ni grains.

Bulk compositions

Ca-Al-rich inclusions and chondrules in ordinary chondrites have similar bulk compositions to those in CV chondrites such as Allende^{8,19} (Table 1). This is illustrated in Fig. 3 where Ca-Al-rich chondrules and inclusions, in terms of CaO and Al₂O₃, plot well within the field defined for such objects from Allende¹⁶ and away from the Mg-Fe-rich chondrules^{19–21}. Most Ca-Al-rich chondrules plot rather parallel and close to, but certainly not on, the cosmic Ca/Al wt ratio line of 1.10 (ref. 22). Ca-Al-rich inclusions, on the other hand, tend to decrease in CaO with increasing Al₂O₃ and trend away from the cosmic Ca/Al abundance line, similar to what had previously been observed for such objects from carbonaceous chondrites^{7,16}. MgO contents of Ca-Al-rich chondrules and inclusions from ordinary chondrites are similar to such objects from carbonaceous chondrites, whereas SiO₂ tends to be higher in objects from ordinary compared with those from carbonaceous chondrites (Fig. 4). Also, most CAIs of carbonaceous chondrites have much lower Na₂O, K₂O and FeO contents than those of ordinary chondrites. Specifically, we note the high Na₂O contents of Ca-Al-rich chondrules (0.34–7.4, average 2.4 wt %) and, particularly, of the Ca-Al-rich inclusions (1.8–7.6, average 5.3 wt %). Such high Na₂O contents are only observed for the coarse-grained¹⁹ (average 4.3 wt %) and fine-grained (type F) inclusions⁸ (average 4.5 wt %) in Allende (Table 1). The average K/Na wt ratios are about 50% lower for the Ca-Al-rich chondrules (that is, 0.05) than for the inclusions (that is, 0.10, exclusive no. 22). The K/Na ratio for Ca-Al-rich inclusions approaches that of CI chondrites (that is, the cosmic wt ratio, ~0.12). Finally, we did not find melilite, common in CAIs of carbonaceous chondrites, in the Ca-Al-rich objects described here; however, recently, we found melilite in a blue, irregularly-shaped inclusion in the Semarkona LL3 ordinary chondrite.

Crystallization sequence

The Ca-Al-rich chondrules clearly crystallized as independent, freely-floating molten droplets. The chondrule from Dimmitt

(Table 1, no. 1), for example, consists of fassaite, spinel, plagioclase (An_{80–86}) and minor olivine (Fo_{73–78}) and Ca-rich pyroxene, embedded in a microcrystalline to glassy mesostasis (Fig. 1). Olivine and Ca-rich pyroxene occur as small ($\leq 10 \mu\text{m}$) grains at the chondrule rim, and when they crystallized relative to the other phases in the chondrules is unresolved. However, we suggest that the euhedral spinel grain crystallized from the melt before the main phase, fassaite, began to crystallize radially from the rim into the core of the chondrule. Plagioclase nucleated on the fassaite crystals just before solidification of the microcrystalline to glassy, CaO-rich mesostasis. Other Ca-Al-rich chondrules consist of early crystallized, skeletal (rapidly grown) fassaite laths or small, skeletal olivine crystals, embedded in a CaO-rich, probably feldspathic, fine-grained mesostasis.

The formation of the Ca-Al-rich inclusions is more difficult to decipher. We found no clear indication that these inclusions crystallized from a melt. It seems that pre-existing dustballs of refractory material are packed together to form the irregularly-shaped inclusions. Alkalis and FeO probably entered the inclusions after condensation but before accretion of the meteorite. When the rims of Ca-rich pyroxene and/or fassaite formed is unknown.

The discovery of a Ca-Al-rich, irregularly-shaped inclusion in a chondrule-like object as described above is important for considerations on the origin of Ca-Al-rich chondrules. The host chondrule material, although rich in refractory elements, has clearly been molten, as probably did the mesostasis of the irregularly-shaped inclusion. This is indicated by partial resorption of the hibonite-spinel assemblage by the mesostasis, as well as by the lack of small cavities within the mesostasis which are common in most irregularly-shaped Ca-Al-rich inclusions. We conclude that the inclusion within the chondrule-like object represents relict material that melted only partly when the chondrule-like object was melted. It thus provides additional evidence for our suggestion that Ca-Al-rich chondrules formed by the melting of pre-existing solid material.

Conclusions

We have found Ca-Al-rich objects (chondrules and inclusions) in all 11 ordinary chondrites we investigated. We conclude that such objects are much more widespread in ordinary chondrites than was previously thought and predict that careful study will probably reveal their presence in most, if not all, type 3 ordinary chondrites. The widespread occurrence of Ca-Al-rich objects in ordinary and carbonaceous chondrites and the textural similarities of Ca-Al-rich and common Mg-Fe-rich chondrules adds additional evidence to the suggestion of Scott *et al.*¹⁷ that

ordinary and carbonaceous chondrites and their constituents have related origins and that CAIs and chondrules are primitive objects that formed by related processes.

The high-temperature minerals in Ca-Al-rich chondrules from ordinary chondrites clearly crystallized from a melt. In an equilibrium condensation scheme from a nebula of solar composition, oxides and silicates of Ca, Al and Ti should dominate at temperatures above about 1,500 K (ref. 23). These condensates should be free of Na and K at temperatures above 1,000 K. Since Ca-Al-rich chondrules are usually rich in Na and K it is clear, in conjunction with the textural evidence presented above, that they are not simple, direct equilibrium condensates but must have had a more complex origin. We rule out that Na and K entered these objects by diffusion after condensation and solidification, since no marginal Na-gradients were observed. We suggest that the Ca-Al-rich chondrules formed by total melting and subsequent crystallization of heterogeneous, submillimetre- to millimetre-sized dustballs consisting of both high-temperature, Ca-Al-rich and lower-temperature, Na-K-rich components as is indicated by the presence of relict material in one chondrule-like object. We argue that these components originally formed at different temperatures and were then mixed in different proportions before total melting and crystallization of Ca-Al-rich chondrules.

Alternatively, the chondrules may have formed by diffusion of K and Na into still liquid drops of high-temperature condensates, an origin we do not favour because it would require rather contrived processes and physical settings. Thus, we suggest that the genesis of the Ca-Al-rich chondrules was not unlike that which has been proposed previously for the origin of the common Mg-Fe-rich chondrules²⁴, except that the starting components of the former were dominated by Ca-Al-rich materials.

Irregularly-shaped Ca-Al-rich inclusions probably formed by agglomeration mainly of refractory dustballs and low-temperature, Fe-, Na- and K-rich material. Secondary heating caused diffusion and reaction between low- and high-temperature components of the inclusions. Some of these objects were probably never molten, whereas others show at least indications for melting in part.

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Growth of chromosome ends in multiplying trypanosomes

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Some of the genes for the variant surface glycoproteins of trypanosomes are located close to a discontinuity in the DNA, presumably a chromosome end. We show here that DNA fragments containing these telomeres increase in length in multiplying trypanosomes at a rate of about 10 base pairs per division. We argue that chromosome growth may not be restricted to trypanosomes and could explain the heterogeneity of telomeric DNA fragments observed in some other organisms.

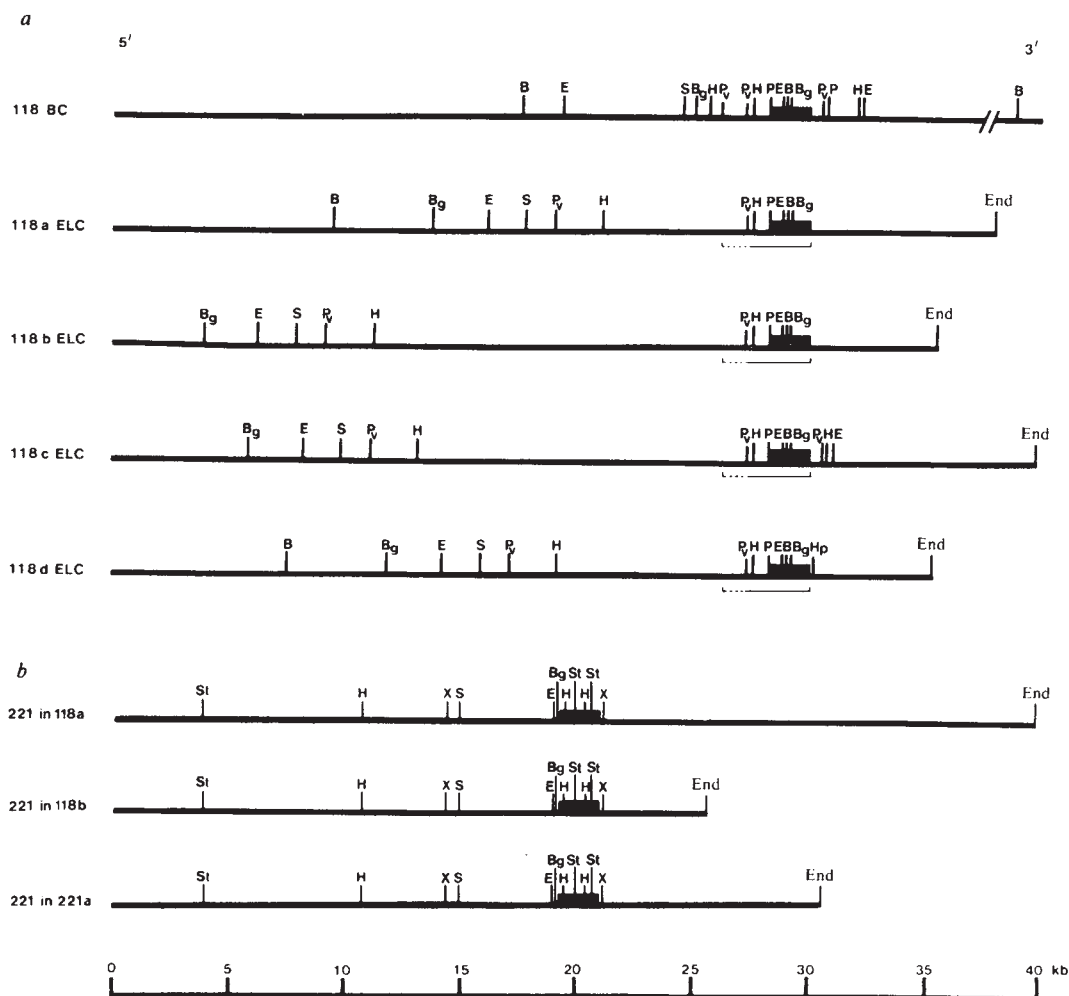
THE presence of linear DNA molecules in eukaryotic chromosomes poses two problems. First, duplex ends are recombinogenic^{1,2} and chromosome ends must, therefore, be marked to distinguish them from DNA breaks. How this is done is still uncertain. Subterminal simple repetitive sequences³⁻¹³ and a terminal hairpin^{3-5,10,14} may be important landmarks. Second, all known DNA polymerases are unable to start new DNA chains without priming. If an RNA primer is used at the 5' end, primer removal results in a gap that cannot be filled without special tricks¹⁵. Three types of strategy are used by viral DNAs to solve this problem: the two strands may be covalently continuous, allowing replication 'around the end'^{10,14}; terminal

repeats may be used to fill up incomplete ends via circularization or concatemer formation^{15,16}; or the priming problem may be solved by a protein that provides the priming deoxynucleotide^{17,18}. Which of these strategies is used by the linear molecules present in eukaryotic chromosomes is still a matter of conjecture¹⁹⁻²¹, based mainly on the analysis of the short linear ribosomal DNAs and amplified macronuclear DNA molecules from primitive eukaryotes^{3-9,12,22}. The lack of probes for chromosome ends has precluded verification of the models proposed.

During our analysis of the DNA rearrangements controlling the expression of surface antigen genes in trypanosomes, we have found that some of these genes are close to discontinuities in DNA, presumably chromosome ends²³. We show here that these ends grow as trypanosomes multiply; we consider possible

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Fig. 1 *a*, Physical maps of the VSG 118 basic copy (BC) gene and the expression-linked copy (ELC) genes from four independently arisen trypanosome clones that make VSG 118 (from ref. 30). The main exon of the 118 gene is indicated by a box, the 35-bp mini-exon donated by the expression site (see text) has not yet been localized precisely, but lies 5' of the upstream *EcoRI* (E) site. The lines beneath the ELCs indicate the segment copied from the BC. 'End' indicates a putative chromosome end (see text). The additional restriction sites in the ELC of variant 118c behind the gene are present in an unrelated DNA element that has moved into the expression site of this variant³⁰. *b*, Restriction maps of the single gene for VSG 221 in trypanosome clones 118a and 118b (both expressing VSG 118) and in clone 221a which expresses the VSG 221 gene (modified from ref. 34 with additional unpublished results). Abbreviations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; St, *Stu*I; X, *Xba*I.



explanations for this phenomenon and we must have evidence that elongation of chromosome ends might not be restricted to trypanosomes.

Variant surface glycoprotein genes

The antigenic determinants detected by host antibodies on living African trypanosomes reside in a single protein, the variant surface glycoprotein (VSG)²⁴. One trypanosome can make more than 100 different VSGs that have no surface-exposed determinants in common²⁵. By repeatedly switching the VSG produced, the trypanosome population can escape complete destruction by host antibodies.

The expression of most VSG genes requires their duplicative transposition into an expression site²⁶⁻³⁰. Entry of a gene displaces the preceding gene from the expression site, probably by a mechanism akin to gene conversion. Before transposition, the VSG gene is incomplete, because it lacks a 35-nucleotide sequence found at the 5' end of mature VSG messenger RNAs (mRNAs) in our trypanosome strain³⁰⁻³³. This mini-exon is provided by the expression site and spliced onto the body of the mRNA encoded within the transposed segment^{31,33,34}. Our results are compatible with the presence of only one functional expression site per nucleus, but this remains to be proven.

The expression site has two unusual properties, illustrated in Fig. 1. First, the transposed gene copy (called the expression-linked copy, ELC) is flanked by long DNA segments that are not cut by restriction endonucleases^{35,36}. These segments change in size as genes are exchanged in the expression site^{30,37}. We attribute the lack of restriction sites to the presence of short, repetitive sequences. The strong selection against recombinant DNA containing these sequences in *Escherichia coli*³⁸ has precluded a direct analysis. Second, the 'barren' region downstream of the transposed gene ends abruptly at a position

where at least 17 restriction enzymes appear to cut³⁶. This point is preferentially attacked by the exonuclease *Bal*31 in long DNA and we have proposed that it represents a duplex discontinuity in the DNA, presumably the end of a chromosome²³. Direct verification of this conclusion by cytological hybridization is not possible, because trypanosomes do not have condensed chromosomes in any phase of their cell cycle.

Gene duplication is not a prerequisite for the activation of all VSG genes, because a subset of genes can be switched on without change in copy number^{27,39-42}. It has recently become clear that genes of this subset always reside at the end of a DNA molecule, that is, upstream of a barren region that ends in a restriction site cluster, preferentially attacked by *Bal*31 (refs 34, 42-44). The 221 gene, illustrated in Fig. 1b, belongs to this subset. Although this gene can be activated without duplication, the 221 mRNA nevertheless has the same 35-nucleotide sequence at its 5' end as the other VSG mRNAs in our trypanosome strain³² and these 35 nucleotides are not contiguously encoded with the remainder of the mRNA sequence³⁴. We therefore think that these telomeric VSG genes are activated by a chromosome end exchange between the chromosome carrying the telomeric gene and that carrying the expression site³⁴. In this way the telomeric gene will be hooked up to the expression site promoter-mini-exon region and the gene present in the expression site would be disconnected. This speculative model, which remains to be critically tested, explains how a single expression site might control the expression of both classes of VSG gene.

Chromosome end growth

The barren regions that flank the transposed gene in the expression site and the telomeric 221 gene vary in size in different trypanosome variants (Fig. 1). We have attributed this

to local deletions and duplications in repetitive DNA³⁷. Such DNA rearrangements might either be coupled to the gene switching process or arise spontaneously as trypanosomes multiply. To decide between these alternatives, we have grown trypanosome variant 118b for up to 340 generations in mice. At intervals the trypanosomes were amplified in rats and the DNA was analysed by blot hybridization. The results are shown in Fig. 2. In Fig. 2a radioactive complementary DNA (cDNA) probe specific for the 118 VSG gene is hybridized to a blot of size-fractionated *Bgl*II digests. Because *Bgl*II cuts the gene, four fragments are expected; in fact, only three are seen because the 3' basic copy (BC) and the 5' ELC fragments co-migrate at 25 kilobases (kb). The striking result, however, is that the 7.0-kb fragment containing the 3' end of the ELC gene increases in length as the trypanosomes multiply. Analogous results were obtained with *Pst*I and *Eco*RI digests (not shown). As these trypanosomes continue to make VSG 118, the ELC itself must be intact. This localizes the length increase in the region between gene and chromosome end.

To test what happens to the telomeric 221 gene, the 118 probe was melted off and the blot was rehybridized with a 221 gene probe. As *Bgl*II does not cut the 221 gene, only a single fragment hybridizes and this increases in length with the number of trypanosome generations. Additional digests also allowed the localization of the increase in length to the area between gene and chromosome end. Moreover, Fig. 3 shows that the shifting fragment is shortened by digesting long trypanosome DNA with *Bal*31 before the restriction enzyme digestion. We therefore conclude that the chromosome termini of dividing trypanosomes grow.

Figure 4 presents the estimated size of the terminal fragments shown in Fig. 2. The growth rate of both telomeres seems to increase with time and to be 50% higher for the ELC telomere (10 base pairs (bp) per generation) than for the 221 gene telomere (7 bp per generation). These differences are not merely artefacts of the electrophoretic procedure used. Growth of the

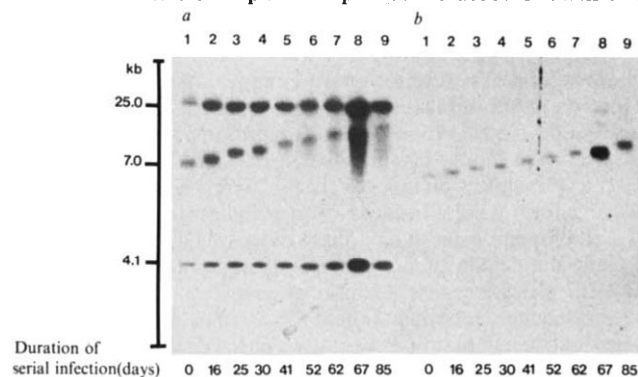


Fig. 2 Autoradiograms of Southern blots showing the increasing size of telomeric DNA fragments in multiplying trypanosomes. Trypanosomes of variant 118b were grown in 'Swiss random' female mice for 85 days (equals 340 generations assuming an average generation time of 6 h) by serial passaging of 10^5 trypanosomes to a new mouse every 3 or 4 days. Antigenic homogeneity of the trypanosome population was checked at regular intervals by indirect immunofluorescence⁴⁷. To obtain sufficient material for DNA analysis, blood from the infected mice was used to infect adult male Wistar rats. The time spent by the trypanosomes in the rat (3-4 days) was added to the total time spent in successive mice to obtain the duration of the serial infection in days indicated below lanes 1-9. *a*, Autoradiogram of a Southern blot containing *Bgl*II digests of the serial DNA samples after hybridization with a ³²P-labelled VSG 118 cDNA probe (TcV118.2; ref. 35). The DNA was digested with restriction endonuclease *Bgl*II, size fractionated in 0.7% agarose, transferred to nitrocellulose filters, hybridized, washed and autoradiographed as described previously³⁵. A *Hind*III digest of phage λ DNA was used as molecular weight standard. *b*, The same blot as shown in *a* after removal of the 118 probe and rehybridization with a ³²P-labelled VSG 221 cDNA probe (TcV 221.5; ref. 48). See Fig. 1 for the *Bgl*II maps of the 118 and 221 genes.

221 gene telomere was also slower in three other digests, including a *Sac*I digest in which the 221 gene telomere migrated more slowly than the ELC gene telomere (not shown). The differences persisted in 0.3% agarose gels, run at low voltage gradients and in denaturing (alkaline) gels. They might be due to a difference in telomere structure. The increasing growth rate of both telomeres shown in Fig. 4 could be due to selection of faster-growing trypanosomes. A growth rate of about 10 bp per generation was also found for a third trypanosome telomere, but this has not been studied in detail.

Obviously, the trypanosomal chromosome ends do not continue to grow to infinite length. The maximal size observed in our variants is the 35-kb barren region downstream of the 221 gene in variant 121a (unpublished result). We do not know what induces shortening *in vivo*, but we have observed shortening in two types of experiment: first, the terminal segment flanking the 118 ELC becomes increasingly heterogeneous as the trypanosomes multiply (see Fig. 2a). As this does not occur with the segment flanking the 221 gene (Fig. 2b) in the same DNA samples, the instability may be related to gene expression. Second, we have observed large deletions in chromosome ends when trypanosomes were put through heat stress. Figure 5a shows the effect of the trypanosome cloning procedure (which involves the manipulation of trypanosomes under a microscope)

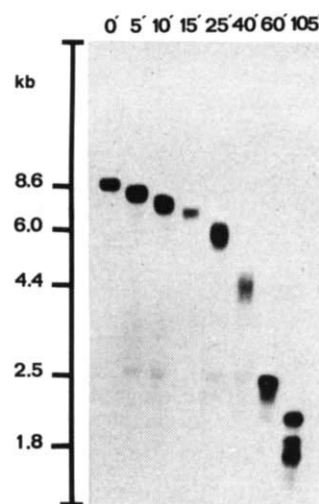


Fig. 3 Autoradiogram of a Southern blot of trypanosome DNA showing that the growing chromosome end downstream of the 221 gene is shortened by *Bal*31 nuclease treatment. 30 μ g of the day 85 DNA sample (see Fig. 2) was incubated with 2.5 U *Bal*31 nuclease²³ (New England Biolabs) for the times (min) indicated. The DNA samples were then extracted with buffered phenol, precipitated with ethanol and digested with restriction endonuclease *Eco*RI. Further handling of the digests was as in Fig. 2b. See Fig. 1 for the *Eco*RI map of the 221 gene.

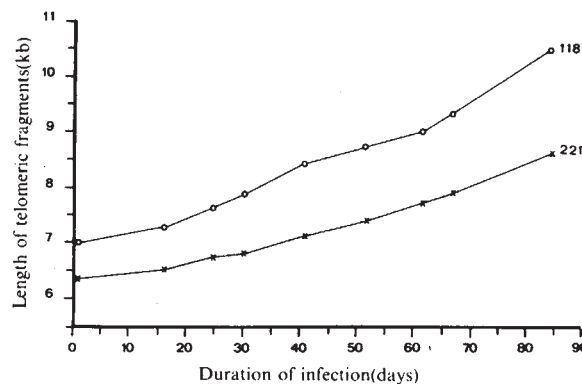


Fig. 4 Graph showing the size of the telomeric *Bgl*II fragments containing the 3' part of the 118 ELC (top) or the 221 gene (bottom) as a function of the duration of the serial infection. The fragment lengths are taken from Fig. 2.

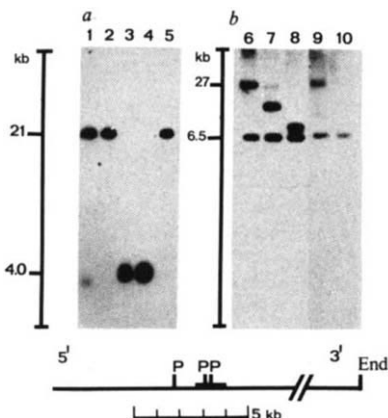


Fig. 5 Deletions in the DNA segment downstream of the 221 gene generated during cloning of trypanosomes or induced by a heat shock. The autoradiograms show Southern blots of size-fractionated *Pst*I digests of 10 different DNA samples, after hybridization with a VSG 221 cDNA fragment that contains part of the 221 gene 3' of the two gene-internal *Pst*I cleavage sites. The *Pst*I map of the 221 gene is shown beneath the autoradiograms. Lanes 2–5 contain four trypanosome clones obtained from the uncloned trypanosome population analysed in lane 1. Trypanosome clones were prepared by injecting single parasites into irradiated mice as described previously⁴⁹. Lanes 6–8 show the hybridization patterns of DNA from three subclones of trypanosome clone 221b, which expresses the 221 gene. In these DNAs, two fragments hybridize, as activation of the 221 gene in this variant was accompanied by the formation of a VSG 221 ELC (unpublished result). To analyse the effect of a heat shock, 150 trypanosomes of variant 221b were incubated for 2 h in mouse blood on ice (as during cloning) and then injected either directly (lane 9) or after a 3-min heat shock at 45 °C (lane 10) into mice. The resulting trypanosome populations were amplified in rats for DNA isolation.

on the length of the telomere flanking VSG gene 221. The initial uncloned population in lane 1 gives a homogeneous band at 21 kb with a minor component at 3.5 kb. Two of the four clones isolated from this population have retained the 21-kb band (lanes 2 and 5); in two other clones (lanes 3 and 4) this has been replaced by a 4-kb band. As these clones were randomly picked, the cloning procedure has in this case resulted in the deletion of 17 kb from the 221-associated chromosome ends in two out of four trypanosomes.

Figure 5b shows an experiment with a trypanosome population expressing the 221 gene. In this variant 221b, the 221 gene is activated via the formation of an ELC, resulting in two telomeric fragments that hybridize to the 221 cDNA probe. Subcloning of this population shows that the longer telomere is unstable in the cloning procedure, whereas the shorter telomere is not (lanes 6–8). Lanes 9 and 10 show a mock cloning experiment. Trypanosomes from the same population used to isolate the clones shown in lanes 6–8 were either kept on ice for 2 h (lane 9) or kept on ice followed by heating for 3 min at 45 °C (lane 10). The heat shock leads to the replacement of most of the 27-kb band by weak bands that migrate faster. Again, the 6.5-kb telomere is not affected. These results suggest that increasing length makes the end increasingly vulnerable to internal deletions and that these could be triggered by stress. As the telomeres probably contain palindromic sequences (see below), the deletions might result from cruciform formation and excision of the cruciform arms.

Implications

Whatever the explanation for the increase of the telomeric fragment size with trypanosome multiplication, it is difficult to see how such regular lengthening could occur in the middle of DNA molecules. We regard our findings, therefore, as strong support for the tentative conclusion^{23,43} that the duplex discontinuities identified by restriction enzyme and *Bal*31 digestion are indeed chromosome ends.

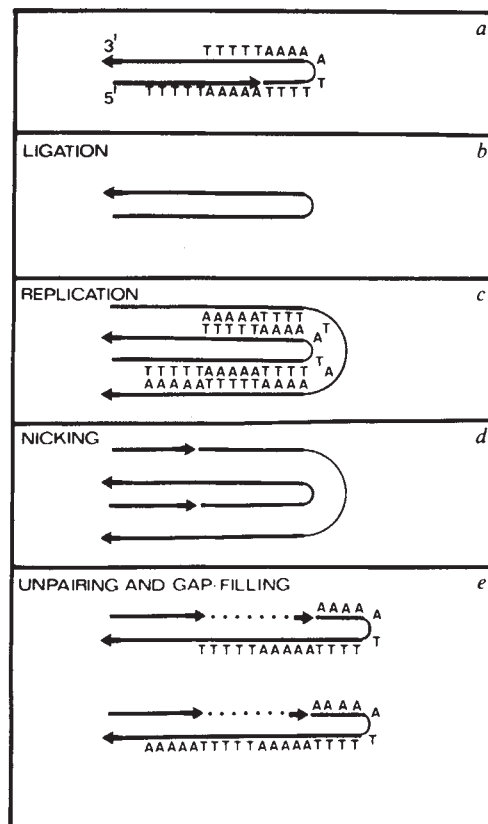


Fig. 6 Speculative model explaining the growth of chromosome ends during DNA replication (modified from ref. 20). In interphase trypanosomes the telomere is assumed to end in a hairpin loop with at least one subterminal nick in one strand (a). During the S phase, the two DNA strands are covalently joined (b) to allow DNA replication 'around the end' (c). Subsequent nicking (d) of two DNA strands is followed by the separation of the two chromosomes and filling of the gap (e). This results in two chromosomes that have grown by 5 bp in the example depicted. The position of the nick determines the rate of chromosome elongation. No information is available on the base sequence of the telomere and the repeat size and sequence shown here were arbitrarily chosen.

Although we have analysed only three chromosome ends, it is highly probable that similar expansion and contraction processes occur at other chromosome ends in trypanosomes. Williams and co-workers^{39–42} have studied three other VSG genes that lie adjacent to chromosome ends. In each case, size variations were observed in the segment flanking the gene and these can easily be explained by the processes described here. We expect, therefore, that the regular size increase of terminal fragments in dividing trypanosomes may be used as an additional criterion to identify telomeric VSG genes. The corollary is that size alterations of trypanosome restriction fragments cannot be equated with gene rearrangements³⁹, unless the presence of an adjacent chromosome end is ruled out.

Finally, we note that the alterations observed here cannot explain the size variations of the barren region upstream of the ELC (see Fig. 1). The 25-kb fragment containing this area changes neither its size nor its homogeneity in the course of 340 trypanosome generations (Fig. 2) and this has been confirmed in other digests in which ELC and BC fragments do not co-migrate (not shown). The alterations that occur in this area when the gene in the expression site is displaced take less than 100 generations and it is therefore likely that these changes are associated with gene switching. If upstream changes are induced by switching, some of the downstream alterations superimposed on chromosome lengthening may also be related to gene switching. However, we cannot verify this, because switching cannot be induced and occurs at a low rate (10^{-4} – 10^{-5} per division).

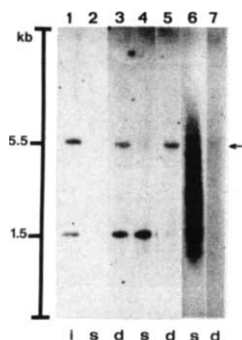


Fig. 7 Autoradiogram showing the presence of rapidly renaturing 'snap-back' DNA in the telomeric DNA fragment containing the 3' part of the VSG 221 gene. 5 μ g of variant 118b DNA (day 0) was digested with endonuclease *Pst*I. After phenol extraction and ethanol precipitation, the DNA was resuspended in 100 μ l 0.12 M sodium phosphate buffer (pH 6.8). The sample was heated for 5 min at 100 $^{\circ}$ C and applied to a 0.25-ml hydroxyapatite column (Biogel HTP, DNA grade) at 60 $^{\circ}$ C as described elsewhere⁵⁰. The column was washed with four 0.25-ml aliquots of 0.12 M phosphate buffer to elute single-stranded DNA and with four 0.25-ml aliquots of 0.40 M phosphate buffer to elute double-stranded DNA. Salmon sperm DNA was added to 10 μ g ml⁻¹ and each fraction was passed over a 1-ml Sephadex G-50 column to remove phosphate by the centrifugal method described by Penefsky⁵¹. The DNA was recovered by ethanol precipitation, dissolved in 0.1 M NaOH, size-fractionated by gel electrophoresis through a 0.7% alkaline agarose gel⁵² and analysed by blot hybridization. A second sample of the *Pst*I digest was taken through the entire procedure, but without prior heat denaturation of the DNA. The recovery of DNA was more than 80% as judged from the recovery of an aliquot of added radioactively labelled DNA. The autoradiogram shows the peak fraction of the 0.12 M and the 0.40 M phosphate buffer elution of the native (lanes 2, 3) and the heat-denatured DNA samples (lanes 4–7). 1 μ g of the *Pst*I digest of input variant 118b DNA is shown in lane 1. Lanes 1–5 were first hybridized with the TcV221.5 cDNA probe to detect the 1.5-kb 5' and the 5.5-kb 3' 221 gene fragment. After removal of the probe, lanes 4 and 5 were rehybridized with a cDNA fragment covering the 3'-most 350 bp of the 221 gene and the resulting blot is shown in lanes 6 and 7. Abbreviations: i, input DNA; s, single-stranded (0.12 M phosphate buffer) fraction; d, double-stranded (0.40 M phosphate buffer) fraction. The arrow indicates a weak band in lane 7 at the position of the telomeric fragment.

Mechanism of chromosome lengthening

It has thus far been impossible to clone the barren regions flanking the chromosome ends in trypanosomes (see ref. 38). Because the concentration of ends is too low for direct analysis, we can only speculate on the process responsible for the increase in length of terminal fragments. Formally, we cannot exclude several far-fetched possibilities: the length increase might be due to internal duplications, to unequal cross-overs, or to end-to-end attachments of chromosomes, the length increase in one end being balanced by shortening of the partner. We shall only consider here, however, the one plausible explanation—that these chromosomes grow by the addition of about 10 bp at the very end during DNA replication.

There are two models for the replication of chromosome ends that can be modified to account for chromosome lengthening: the terminal palindrome model^{19–21} illustrated in Fig. 6 and the loop-back model^{45,46}. Both models postulate the existence of a sequence-specific endonuclease that nicks a characteristic repeat. Displacement of the nick by one repeat unit will result in chromosome lengthening by one repeat per division. As denatured telomeric DNA migrates in alkaline agarose gels as a linear molecule with separable strands²³, we assume in Fig. 6 that there is a subterminal nick in the telomere when replication is not in progress.

The modified palindrome model predicts that chromosomes will grow by addition of palindromic DNA segments. When these segments are heat-denatured and rapidly cooled, they should snap back to form largely duplex DNA that will be

retained on hydroxyapatite in 0.12 M phosphate. Figure 7 shows an experiment in which this prediction was verified for the telomere flanking the VSG 221 gene. After denaturation, the fragment containing the telomere is retained on hydroxyapatite (lane 5) whereas the fragment containing the 5' half of the gene remains nearly completely in the single-stranded DNA fraction (lane 4). The controls in lanes 6 and 7 show that moderately repetitive DNA does not renature in the conditions of this experiment. The 3' half-gene 221 probe, which cross-hybridizes to the 3' ends of many VSG genes, hybridizes nearly exclusively with a DNA smear (lane 6) in the denatured DNA fraction; the very weak band in the renatured fraction (arrow) is probably the 221-containing snap-back telomere. These results are compatible with the modified palindrome model, but also with a loop-back model if the telomeric fragment happens to contain a long palindrome upstream of the segment added on by growth.

Are growing chromosomes unique to trypanosomes?

Because it is known that different strategies exist in nature for getting complete copies of the ends of linear DNA molecules, it is possible that growing chromosomes are a peculiarity of trypanosomes. It is noteworthy, however, that several linear DNAs that replicate in a linear form and from centre to periphery are known to have ragged ends. These include the mitochondrial DNA of *Tetrahymena*⁴⁶, the rDNA of *Tetrahymena*³ and *Oxytricha* and the rDNAs of the slime moulds *Physarum*¹² and *Dictyostelium*¹³. These DNA molecules are present in many copies per cell and synchrony therefore cannot be expected. In all these cases size heterogeneity is much less than that observed for the trypanosome telomeres, but this would only require that chromosome shortening occurs more frequently, reducing the range over which chromosomes will grow.

Although the ciliate rDNA molecules are rather short chromosomes, their telomeres may, in fact, be representative of eukaryotic chromosomes in general. The elegant experiments of Szostak and Blackburn⁴ have recently shown that *Tetrahymena* rDNA telomeres will act as stable telomeres for *Saccharomyces* (yeast) mini-chromosomes. In their remarkable trans-kingdom recombinant, the *Tetrahymena* telomere had regained its size heterogeneity by the time sufficient material had been grown up to analyse it. This suggests that this heterogeneity is an essential feature of chromosome ends in general. The possibility that growing telomeres are a characteristic of chromosomes in many eukaryotes therefore seems worth investigating.

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LETTERS TO NATURE

On the origin of pulsar velocities

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We have recently¹ found the velocities of 26 pulsars from their proper motions as measured by a long-baseline radio interferometer. We now point out that these velocities are closely correlated with either the magnetic dipole moments or the moments of inertia of the pulsars as derived from the slow down of their rotation rates. Contrary to previous reports, we find no relation between the directions of the proper motions and the pulsar spin axes. A brief examination is made of possible explanations of the correlations with velocity and it is suggested that an asymmetry at the time of neutron star formation is responsible. The data do not seem to support the electromagnetic acceleration process suggested by Harrison and Tademaru².

The velocities of pulsars are generally an order of magnitude larger than for most stellar populations. Interferometric measurements¹ of proper motion indicate that pulsars are born within about 100 pc of the galactic plane, with a typical velocity of about 220 km s⁻¹ which they acquire either at birth or soon afterwards. They then rise to heights of several hundred parsecs from the plane before they fade. These data are consistent therefore with births in the supernovae of massive Population 1 stars, although the mechanism for the production of the high velocities is unclear.

All known transverse velocities v_t are shown in Table 1, omitting only PSR1919+21 for which the published measurement depends on rather uncertain¹ timing observations, and including the optical determination for the Crab pulsar by Wyckoff and Murray³. Table 1 also shows the dipole magnetic moment m , as deduced from the rotation period P and its derivative $\dot{P}$, according to

$$m = \left[\frac{3c^3}{8\pi^2} I \dot{P} P \right]^{1/2} = 3.2 \times 10^{37} (P \dot{P})^{1/2} \text{ G cm}^3 \quad (1)$$

which assumes that the moment of inertia of all pulsars is $I = 10^{45} \text{ g cm}^2$ (ref. 4). This relationship depends on the usual assumption that the rotational slow down is due to emission of electromagnetic dipole radiation at the rotational frequency^{5,6}; it refers, of course, to the component of dipole moment perpendicular to the rotation axis. Figure 1 shows that there is a remarkably good correlation between the velocity of a pulsar and its magnetic moment (correlation coefficient 0.63, significant at the 0.1% level).

There is now a substantial body of evidence which shows that the effective magnetic dipole moments of pulsars are decaying on a timescale of <10 Myr (see refs 1, 7–9). Thus the magnetic dipole moments at birth were substantially greater than we observe at present. The present magnetic moment m and initial magnetic moment m_i are related by

$$m^2 = \frac{m_i^2}{(1 + \tau/\tau_B)} \quad (2)$$

where $\tau = P/2\dot{P}$ is the characteristic age and τ_B is the characteristic decay time of the magnetic field. In Fig. 2 the transverse velocities are plotted as a function of the initial magnetic moments calculated for $\tau_B = 8 \text{ Myr}$. The correlation coefficient of v_t against m_i of 0.54 is significant at the 0.5% level. There is a substantial steepening of the relationship between velocity and magnetic moment because most of the low-velocity pulsars are quite old and have suffered more from magnetic field decay. There is still, however, a dearth of low-velocity, high-moment and high-velocity, low-moment objects.

It is possible that the trends seen in Figs 1 and 2 are caused in part by a selection effect. It has been shown⁹ that the luminosities of pulsars are roughly proportional to the squares of their magnetic moments. Pulsars having high magnetic moments are therefore visible in a larger volume of space in the vicinity of the Sun than are pulsars having low magnetic moments. Thus, some high-velocity, low-moment pulsars could have migrated far enough from the galactic plane that the received flux is too small for them to be included in the pulsar

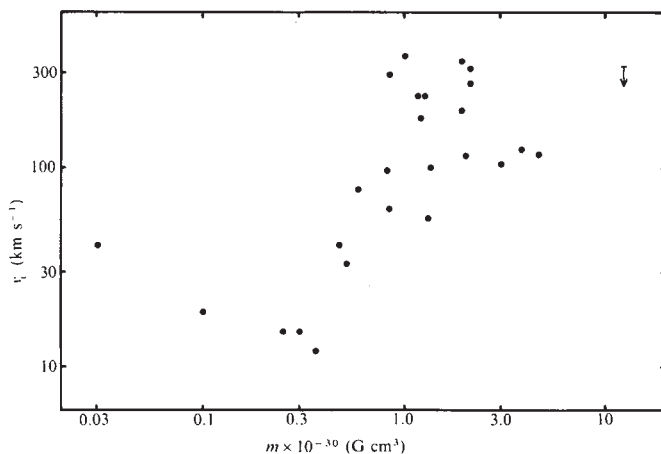


Fig. 1 The transverse velocities of 26 pulsars plotted as a function of the effective magnetic dipole moment obtained assuming a moment of inertia of 10^{45} g cm^2 .

sample. This is supported to some extent by the fact that the lower velocity pulsars in the sample tend to be closer to the Sun and at lower z -distances above the galactic plane² than the higher velocity sample. If, however, the correlations between v_t and m or m_i are not real, then we would expect the missing pulsars in the top left of the diagrams to be otherwise intrinsically the same as those in the sample. An examination of the pulsars in the sample shows that a substantial fraction should then be visible: they are not. In addition, we have not found any selection effect which can account for the absence of low-velocity, high-magnetic moment objects. We conclude that the correlation is not the result of a selection effect.

The correlation in Figs 1 and 2 is between velocity and magnetic dipole moment. However, it is possible that the correlation arises because of a physical relationship between velocity and the moment of inertia of the pulsar. Correlation between pulsar velocity and moment of inertia would occur if all pulsars were given the same momentum impulse at birth, and had the same magnetic dipole moments but had a range of masses. Pulsars of small mass, and therefore small moments of inertia¹⁰, would then have high velocities. Our assumption that all pulsars have the same moment of inertia would then impress the variations in moment of inertia on the interpreted magnetic dipole moments [equation (1)]. The quasi-linear relationship between neutron star mass and moment of inertia in the models reviewed by Canuto and Bowers¹⁰ leads to a relationship of slope 2 in Fig. 2. This is certainly compatible with the data and from the experimental evidence we cannot rule out this possibility. However, a range of neutron star mass of more than an order of magnitude (more than most present models allow) and improbably narrow dispersions in both momentum impulse and magnetic dipole moment at birth are required to explain the observations. We conclude, therefore, that there is a real tendency for the higher velocity pulsars to have higher magnetic fields.

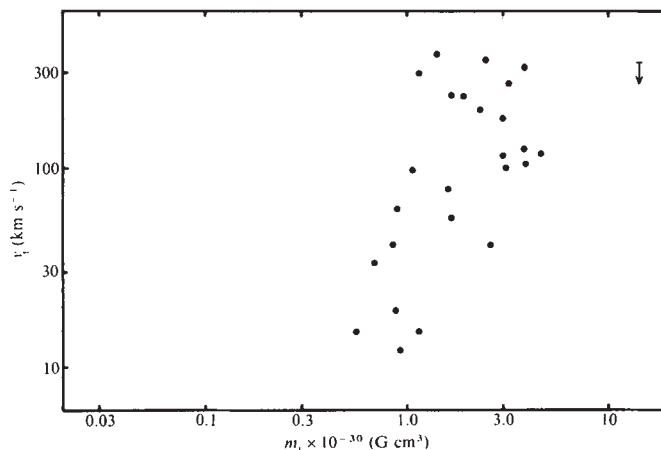


Fig. 2 The transverse velocities of 26 pulsars plotted as a function of the effective magnetic dipole moment at birth, assuming an exponential decay of the magnetic field with a characteristic time of 8 Myr.

In attempting to explain this remarkable observation we have examined the proposal² that pulsars are accelerated to high velocities during the first few months after the collapse of a progenitor star. The acceleration arises from asymmetry in the electromagnetic radiation field of the pulsar resulting from the magnetic dipole field being inclined to the rotation axis and offset from the centre of the pulsar. The magnitude of the final space velocity depends on the magnetic field configuration but, as Helfand and Tademaru¹¹ point out, it is not expected to depend on the magnitude of the magnetic dipole moment. This is because the accelerating force is proportional to the square of the moment but the time for which it acts is inversely proportional to the square of the moment.

Table 1 Observational data on 26 pulsars

PSR	Long. (deg)	Lat. (deg)	Distance (pc)	v_t (km s ⁻¹)	Error	m (cgs)	m_i (cgs)	ψ_v (deg)	Error	ψ_p (deg)	Error	$\psi_v - \psi_p$ (deg)	Error
0301+19	161.1	-33.3	560	100	11	30.13	30.49	171	11	23	4	32	12
0329+54	145.0	-1.2	2,300	229	11	30.09	30.28	124	2	19	4	75	5
0355+54	148.2	0.8	1,600	62	23	29.92	29.95						
0525+21	183.9	-6.9	2,000	141	180	31.09	31.16						
0531+21	184.6	-5.8	2,000	123	38	30.58	30.58	-67	18	139	10	26	20
0611+22	188.9	2.4	3,300	117	63	30.66	30.66						
0809+74	140.0	31.6	170	41	5	29.67	30.42	163	8	153	7	10	11
0823+26	197.0	31.7	710	365	10	29.99	30.16	146	1	48	3	82	3
0834+06	219.7	26.3	430	104	6	30.47	30.59	2	5	67	11	65	12
0943+10	225.4	43.1	560	115	48	30.30	30.47						
0950+08	228.9	43.7	90	15	3	29.39	29.75	26	12	162	4	44	13
1133+16	241.9	69.2	150	264	2	30.33	30.51	-16	1	96	7	68	7
1237+25	252.5	86.5	330	178	6	30.07	30.48	-68	1	167	5	55	5
1508+55	91.3	52.3	730	346	10	30.29	30.39	-133	2	5	5	42	5
1541+09	17.8	45.8	1,300	78	25	29.76	30.21						
1604-00	10.7	35.5	360	12	17	29.56	29.97						
1642-03	14.1	26.1	1,300	294	11	29.92	30.06	121	16	83	10	38	19
1818-04	25.5	4.7	1,500	194	21	30.29	30.36	6	7	83	20	77	21
1929+10	47.4	-3.9	80	33	2	29.71	29.84	64	2	59	2	5	3
1944+17	55.3	-3.5	430	19	8	29.02	29.95						
1952+29	65.9	0.8	200	41	10	28.47	29.93						
2016+28	68.1	-4.0	1,300	15	18	29.47	30.06						
2020+28	68.9	-4.7	1,300	97	18	29.91	30.03	-147	9	176	3	37	9
2021+51	87.9	8.4	680	56	13	30.11	30.22	19	12	29	3	10	12
2217+47	98.4	-7.6	1,500	230	43	30.09	30.22	-158	14	82	10	60	17
2303+30	97.7	-26.7	1,900	316	54	30.33	30.58						

The galactic longitude and latitude and distance are given, together with the present magnetic dipole moment m and the initial dipole moment m_i obtained assuming a magnetic field decay time of 8 Myr. v_t and ψ_v are the magnitude and celestial position angles of the transverse velocity taken from ref. 1 except for PSR0531+21 which is taken from ref. 3. ψ_p is the celestial position angle of the linear polarization at the centre of the pulse obtained from ref. 15. For PSR1237+25, 90° has been added to ψ_p because of a probable rapid position angle sweep of 180° near the centre of the pulse¹⁴.

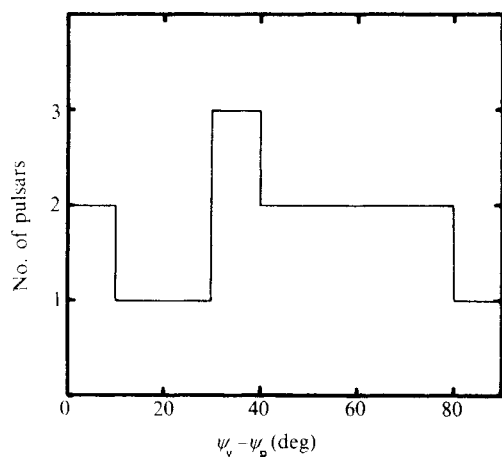


Fig. 3 The histogram of the differences between the position angles of proper motion and linear polarization, $\psi_v - \psi_p$. 16 pulsars are included, all having errors in the difference angle of $\leq 25^\circ$.

Helfand and Tademaru¹¹ first noted the trend in Fig. 2 in a smaller data set. They suggested that there are two classes of pulsar: a high-velocity class born from single stars or weakly bound binaries and accelerated by the mechanism described above to final velocities independent of magnetic field, and a low-velocity, low-field class born in close binaries (see ref. 12). Having two classes of pulsar with differing typical velocities and magnetic fields would explain the apparent correlation in Figs 1 and 2 and allow the higher velocities to be independent of magnetic field. However, the previous clear separation of the classes is not supported by the data on the greater number of sources in the present sample, although it cannot definitely be ruled out.

A further prediction¹³ of the acceleration theory is that the velocity direction should be related to the rotation axis of the pulsar which can be determined from observations of the linear polarization of the radio emission. Tademaru¹⁴ has suggested that the difference in position angle should be predominantly near 0° or 90° depending on the emission mechanism and the inclinations of the rotation and magnetic field axes to the line of sight. He found a bimodal distribution peaked at 0° and 90° for data of dubious quality. Figure 3 shows the histogram of position angle differences between the velocity vector ψ_v and the intrinsic linear polarization position angle ψ_p using the later, higher quality data for 16 pulsars taken from refs 1, 15, which are summarized in Table 1. The pronounced peaks found by Tademaru¹⁴ near 0° and 90° are not present. There is, however, a tendency for lower velocities to be associated with smaller position angle differences and higher velocities with larger differences. Because of the small number of objects in the sample, this trend is not statistically significant. We conclude that the data now available do not support the electromagnetic acceleration theory for the origin of the high space velocity of pulsars.

Two other sources for the high pulsar velocities have been suggested: evolution of close binary systems¹⁶ and asymmetry in the supernova event¹⁷. Various evolutionary sequences¹² have been suggested for close binary systems, many of which result in the collapse and supernova of the more massive star and the production of a pulsar. The supernova also causes a substantial and sudden mass loss which, even if completely symmetric, can result in the system being disrupted. But while the unbound companions finish with a substantial part of their orbital velocities, we are not aware of any suggestions that the velocity of the pulsar should be related to its magnetic moment. Asymmetry in the collapses and subsequent explosions in supernova events seems also able to produce high velocities¹⁸, even though difficulties have been encountered with some simple models¹⁹. Again it is not evident how the correlation between velocity and magnetic moment would arise, unless the asym-

metry were related in some way to the magnetic field configuration of the progenitor.

Most theoretical models of stellar collapse and supernova explosions are understandably oversimplistic and do not, for example, take rotation or magnetic field into account²⁰. The translational kinetic energy of a typical pulsar is about five orders of magnitude smaller than the kinetic energy of the material ejected in the expanding supernova shell, so that the amount of asymmetry required to produce a typical pulsar velocity seems quite small. The influence of magnetic field on a supernova event looks, at first sight, to be negligible from energy considerations. Nevertheless, if the correlation between velocity and magnetic dipole moment is real, and asymmetry in the supernova event leads to the velocities, then either the magnetic field influences the degree of asymmetry, or else some unknown source of asymmetry influences both the velocity and the residual field of the pulsar.

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Soft X rays and fast atoms as image generators in photoelectron microscopy

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The magnetically collimated photoelectron spectromicroscope (PESM) was initially developed with the He I light source^{1,2}. Although one of us³ has previously mentioned the successful use of other resonance lines, of a soft X-ray source and of metastable atoms as alternative ionizing sources, at that time no electron energy analysis had been performed using the X-ray source to decide in what proportion primary photoelectrons, Auger electrons and inelastically-scattered electrons contributed to the observed images. We now have this information and can compare the performance of these different sources, particularly in the direction of chemical analysis across a complex object. We also report for the first time energy-selected imaging using a fast-atom bombardment source.

Operation of a thermionic emitter-X-ray source, in the high magnetic field (~ 7 T) used by us, displays some unusual features and advantages connected with the possibility of using flux guidance of the exciting electron beam. This topic will be fully described elsewhere. In brief, X rays were generated in anodes placed near the magnet centre and close to the object. Resonance light from a gas discharge and also atoms from a fast-atom

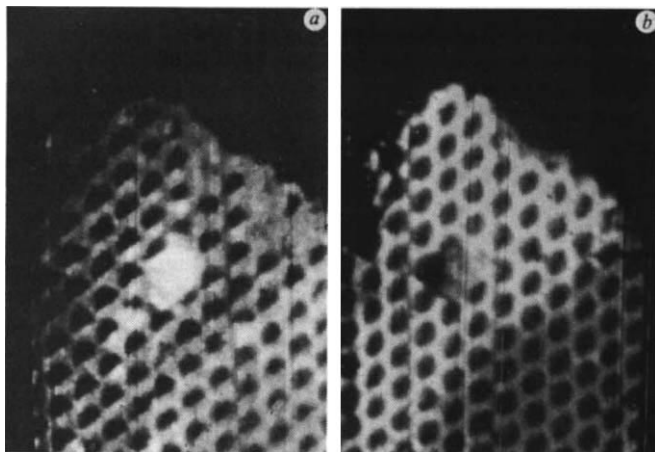


Fig. 1 Emission electron images of a fragment of microchannel plate (hole diameter $\sim 15\ \mu\text{m}$) excited by: *a*, C(K) X rays; *b*, fast (2 kV) Ar atoms.

source entered from the sides. The electron flux to the X-ray anodes passed 1 cm away from the magnetic axis. The electron beams were launched from tungsten filaments, part circles for best mechanical stability in the high magnetic field, positioned behind the object region remote from the anodes to minimize tungsten contamination. In the case of C(K) production (graphite coated Al anode) we obtained bright images with a 4 mA, 2 kV electron beam but usable images could be recorded with electron beams as low as 0.5 mA, 400 V.

Fast neutral atoms (Ar) were introduced at right angles to the magnetic field from an electron oscillator source sited near the radial null point in the radial magnetic field. Those ions which do not undergo charge exchange neutralization are then deflected by the increasing magnetic field as they move to the magnet centre. With this arrangement the source is relatively close (9 cm) to the sample. Alternatively a more conventional saddle-field FAB source (Iontech (UK) Ltd) was placed close to the field axis 50 cm in front of the sample. We have also

obtained images using metastable atoms (Ar and He) effusing from a pin hole source 14 mm from the sample.

We will distinguish contrast mechanisms⁴ which can be classified as primary or secondary in origin. Primary effects relate to the different mechanisms which control the efficiency for the primary conversion stage by which the incident energy liberates an electron (or electrons). For example, the position of absorption edges and energy-dependent cross-section variations. Tuning across an absorption edge should produce sudden contrast changes. Then secondary effects can be discerned arising from the differing probabilities for escape to, and through, the surfaces of varying chemical composition, adsorbed layers and physical structure (crystallite orientation and so on) and will show marked energy dependence. Internal inelastic scattering is likely to be an important factor with energy loss to electronic and phonon excitation, and to vibrational modes in adsorbed molecules. This implies a true 'chemical' contrast reflecting the molecular structure at the surface.

We show first how the image contrast varies with the ionizing source used using no electron energy selection. The test object was a broken fragment of electron multiplier microchannel plate having a hole diameter of $\sim 15\ \mu\text{m}$. The fracture exposed a broken edge in which the treated inner surfaces of the channels were exposed as well as the freshly broken untreated glass surface. The contact face was subsequently gold coated by evaporation and fragments of sodium chloride included.

The relative brightness of the sodium chloride fragments in the C(K) photoelectron image (Fig. 1) is clearly a primary effect the L shell of chlorine (L III 200 eV to L I 270 eV) having a high cross-section for ionization by C(K), 275 eV. In the image using He I generated photoelectrons, selected for the energy band above 10 eV, the sodium chloride fragment is black. More subtle differences, which may be in part secondary in their origin, may be discerned by comparing the four images obtained using C(K) X-rays, fast atoms, He I and H Ly α shown in Fig. 2. Note, for example, differences between the interior of the channels (hydrogen reduced) and the adjacent broken edges (unreduced).

If we restrict the range of energies of the electrons which form the image the dominant mechanism may change. For example, by selecting only low-energy electrons when soft X rays are the ionizing source the escape dependent contrast will

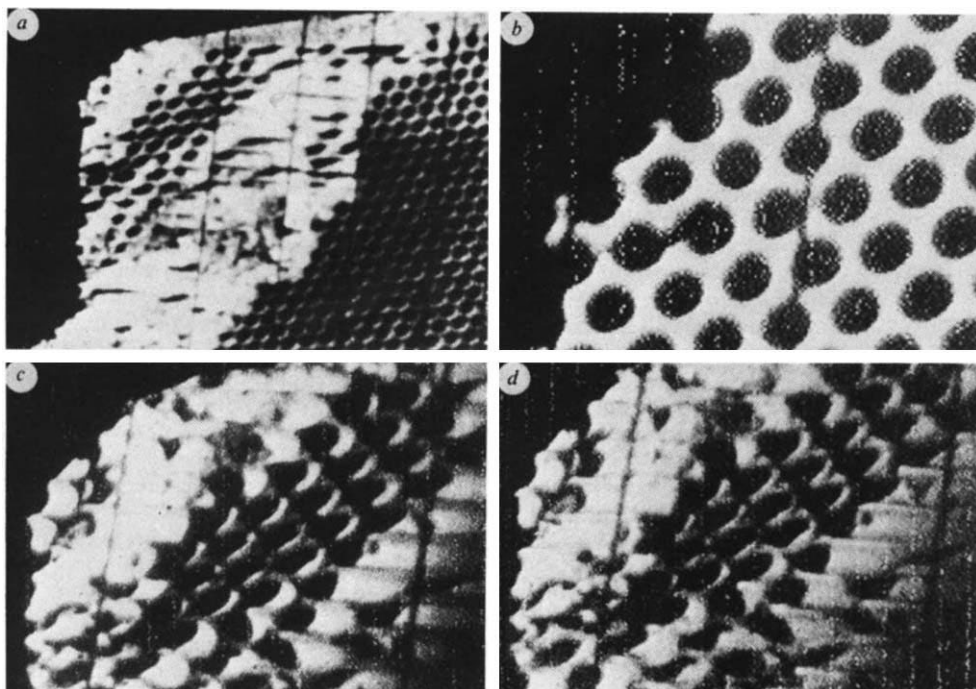


Fig. 2 Emission electron images of a fragment of microchannel plate irradiated with: *a*, C(K) X rays (from above and to the left); *b*, fast (2 kV) Ar atoms (from the right); *c*, and *d* vacuum UV radiation, He I and H Ly α respectively (from the left, see text). Note that the magnification varies.

be dominant. However, at the same time the spatial resolution is affected. The higher-energy sources allow higher electron kinetic energies to appear in the photoelectron spectrum and their larger cyclotron orbits constitute a resolution limit when only these higher-energy electrons contribute to the image. Figure 3 shows an electron microscope gold finder grid over an aluminium surface imaged by bremsstrahlung induced photo- and Auger electrons with the electron energy accepted being varied so as to include either all electrons or only electrons of energy above 800 eV. We note that this loss of resolution may be reduced by removing from a group of electrons of particular energy those whose initial motion is largely transverse to the magnetic field, a procedure we term 'skimming'.

The mechanism of ionization with fast atoms is both energetic neutral atom-surface collisions and surface Penning ionization. In Penning ionization an inert gas atom in a metastable state, which is sufficiently energetic in relation to the surface work function, approaches the surface. An electron from the surface valence band then tunnels through the surface to fill the partially empty ground-state level of the atom. The electron in the metastable level is then emitted. This process is similar to photoemission but involves different selection rules. We have previously reported³ images produced by an excited neutral-atom source which must represent purely Penning ionization. To our knowledge metastable atoms have not previously been used to generate electron images of surfaces. We now show the utility of beams of fast atoms, some of which may be metastable, in generating bright images (Fig. 2*b*). Almost the whole electron flux is at low energy (<1 eV) and the image resolution is at its optimum.

Electron-energy analysis can be performed on a selected feature by isolation of the electron flux in the appropriate image region. Of the possible methods of electron-energy analysis in a strong magnetic field we have found retardation followed by either differentiation or trochoidal deflection by a crossed electric field to be the two most suitable. This allows chemical analysis in PESM using photoelectrons and in certain cases Auger electrons.

The functions of imaging and trochoidal energy analysis can be combined by using a slit to define an image strip and

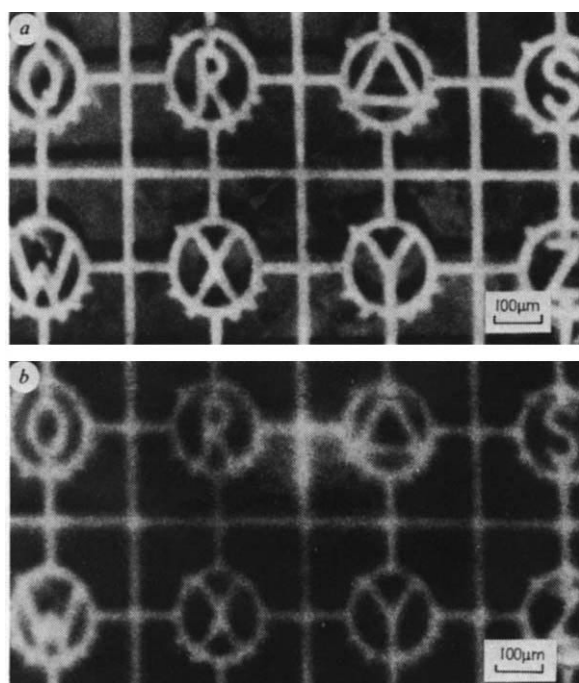


Fig. 3 Soft X-ray photoelectron images of a gold EM 'finder' grid over an aluminium support. *a*, No retardation before the image plane; *b*, -800 V retardation before the image plane (see text).

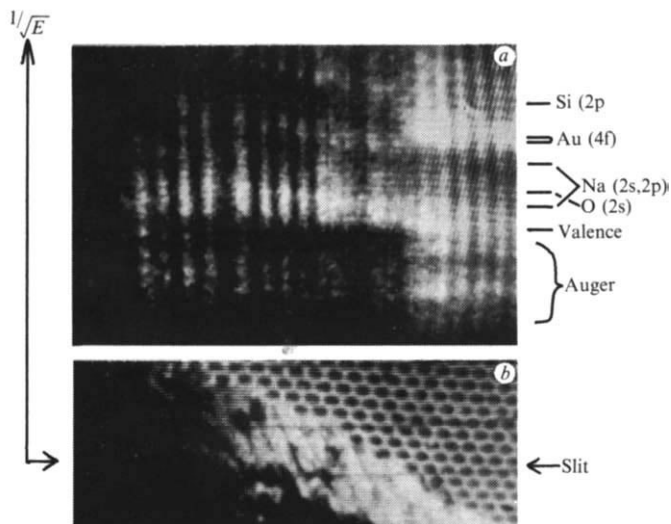


Fig. 4 *a*, Dispersion of the C(K) excited photoelectron emission from a section of microchannel plate under the influence of an electric field at right angles to the magnetic field (see text). A defining slit was placed at the position indicated by the arrows on the locating image; *b*, (excited here by mixed He I and Ar fast atoms). Photoelectron assignments and the presence of Auger electrons are indicated.

subsequent motion in an E field transverse to the (low) B field and parallel to the slit to disperse the spectrum at right angles to it. In the crossed-field arrangement the transverse deflection is inversely proportional to the square root of the electron energy. For a fixed exit aperture the spectrum may be generated by a linear scan of a retarding voltage applied before the transverse field or for highest sensitivity the spatially-dispersed inverse square-root energy distribution is directly obtainable from the image.

This latter method, multichannel energy analysis for a linear array of object points, is illustrated in Fig. 4. The slit selected a strip which included both the gold-coated face of the channel plate sample and a part of the uncoated broken edge. The electron flux was then retarded by 100 V before entering the crossed field region to increase the dispersion. The change of chemical composition as a function of distance is evident both from the changes in the photoelectron energy distribution particularly the Au(4f) to Na(2s) and Si(2p) signals but also in changes in the Auger electrons (see assignments on Fig. 4). Changes in chemical composition can be discerned in distances comparable with the channel diameter (15 μm).

The trochoidal analyser has also been used for single point analysis with a channeltron electron multiplier to detect the transmitted electron count rate. This approach gives a greater dynamic range than is possible by detection of the light intensity at the phosphor screen. The pass energy and the energy resolution can be varied by changing the crossed electric field. The analyser performance has been measured using an electron signal from a thermionic emitter (FWHM probably 0.5 eV or greater). For a transmitted beam energy of 700 eV a FWHM of 1.4 eV has been recorded.

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Atomic diffusion coefficients calculated for transition metals in olivine

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Atomic diffusion in minerals is one of the most important processes for obtaining information on the formation and subsequent cooling histories of minerals or rocks. We have estimated the atomic diffusion coefficients of four transition metals in the forsterite structure on the basis of the potential energy map using the Born–Mayer type repulsive energy term in the WMIN program^{1,2}. The cation was assumed to migrate along the minimum energy pass of the map. We have estimated the activation energies for the M1 cation migration from the height of the saddle point at the pass, and the frequency terms for diffusion from the shape of the energy minimum around the M1 site. Our calculated values are in line with experimental results³. Although the diffusion phenomena give us information about the genesis and cooling histories of the minerals, the atomic diffusion coefficients in geologically important minerals such as pyroxene are often too low to measure experimentally; we show here how our theoretical approach may be applied to such minerals.

Although the chemical zoning, common among rock-forming minerals, is closely related to the atomic diffusion process, the diffusion coefficients in geologically important minerals are too low to be determined experimentally. For example, the Ca atomic diffusion coefficient in pyroxene is estimated to be lower than $10^{-15} \text{ cm}^2 \text{ s}^{-1}$, but its accurate value has not yet been determined experimentally⁴.

We approached the theoretical estimate of the atomic diffusion coefficient by calculating the 'potential energy' map of the olivine structure using the WMIN program of Busing^{1,2} in which the Born–Mayer type repulsive energy is assumed. The atomic diffusion coefficients of four transition metals (Mn^{2+} , Fe^{2+} , Co^{2+} and Ni^{2+}) in forsterite ($\alpha\text{-Mg}_2\text{SiO}_4$) were estimated and compared with those determined experimentally³. The diffusion process in olivine, one of the most important minerals of the Earth's mantle, has been the subject of intensive research, and we felt it would be appropriate to apply our method in the forsterite structure to atomic diffusion process in olivine. The validity of our method may be tested by such comparison.

The expression for the potential energy used in this study is²

$$W = \frac{1}{2} \sum_i \sum_j \left[\frac{Q_i Q_j}{r_{ij}} + f_0 (B_i + B_j) \exp \left(\frac{A_i + A_j - r_{ij}}{B_i + B_j} \right) \right] \quad (1)$$

Table 1 Repulsive parameters A_i and B_i

	A_i^*	B_i	Shifted $A_i^\dagger$	Ionic radius [‡] (Å)
O^{2-}	1.789	0.104	1.364	1.38
Si^{4+}	0.528	0.0149	0.467	0.26
Mg^{2+}	0.788	0.0218	0.699	0.72
Mn^{2+}	1.190	0.0907	0.819	0.830
Fe^{2+}	1.079	0.0790	0.755	0.780
Co^{2+}	0.864	0.0336	0.727	0.745
Ni^{2+}	0.749	0.0118	0.700	0.690

* $f_0 = 1 \text{ kcal mol}^{-1} \text{ Å}^{-1}$.

† $f_0 = 59.9 \text{ kcal mol}^{-1} \text{ Å}^{-1}$.

‡ From ref. 10.

where r_{ij} is the distance between two ions. Q_i is the ionic charge, and we assume that this is a fully ionized model⁵. The second term in square brackets represents the repulsive energy^{1,6,7}. A_i is an ionic radius and B_i is an ionic compressibility. Constant f_0 is taken as $1 \text{ kcal mol}^{-1} \text{ Å}^{-1}$.

We estimated the repulsive parameters A_i and B_i of Mg^{2+} , Si^{4+} , O^{2-} , Mn^{2+} , Fe^{2+} , Co^{2+} and Ni^{2+} ions by employing the refined structural parameters^{8,9} of $\alpha\text{-Mg}_2\text{SiO}_4$ (forsterite), Mn_2SiO_4 (tephroite), Fe_2SiO_4 (fayalite), Co_2SiO_4 and Ni_2SiO_4 . To bring the A_i s to the same scale as the traditional ionic radii¹⁰, an f_0 value of 59.9 was chosen for these olivines⁵ (Table 1). These parameters have been successful in simulating the crystal structures of minerals when employing the energy minimization method of WMIN¹¹.

According to the Eyring theory for the rate process¹², the atomic diffusion coefficient (D) can be calculated from the activation energy and the frequency term for diffusion:

$$D = \lambda^2 \frac{kT}{h} (1 - e^{-h\nu/kT}) e^{-E/N_A kT} \quad (2)$$

where h , k , λ , T and N_A are Planck constant, Boltzmann constant, distance, temperature and Avogadro number, respectively. E and ν are activation energy and frequency for diffusion, respectively. Ohashi and Finger¹³ and Dempsey and Freer¹⁴ have considered the cation diffusion route in olivine. The olivine structure has a zig-zag arrangement of vacant cation sites along the c axis, and the cations are expected to go through this channel to migrate into another site. We have assumed a simple classical model for the atomic diffusion process where the Mg ion is mobile along the valley of the potential energy map in the forsterite structure. The Mg ion moves along the valley, goes over a saddle point (minimum energy barrier) and reaches a neighbouring minimum energy point on the map. The Mg ion will be mobile in the direction where the height of the barrier is low. The magnitude of the energy necessary to go through barrier is defined as the difference of the potential energies which are calculated for the ion located at the octahedral cation site and at the saddle point. We assumed this value

Table 2 Atomic diffusion coefficients, activation energies and frequencies calculated for transition metals in the forsterite structure

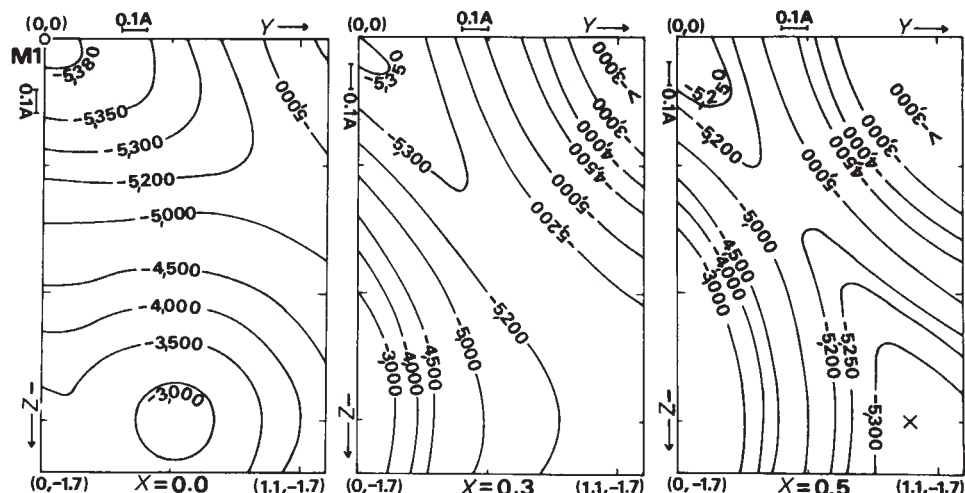
	Mg*	Mn	Fe	Co	Ni
E_0	-5,383.3	-5,308.0	-5,343.3	-5,370.4	-5,386.4
E_s	-5,321.7	-5,245.1	-5,289.1	-5,304.8	-5,319.7
$E (=E_s - E_0)$	61.6	62.9	54.2	65.6	66.7
	(106.2)	(102.6)	(63.2)†	(74.9)	(99.0)
k	286.0	337.0	219.0	340.0	342.0
$\tilde{\nu}$	373.0	269.0	215.0	261.0	262.0
$D_{1,200}$	6.3	3.0	48.0	1.2	0.80
	(0.28)	(6.0)	(24.0)†	(2.2)	(0.47)

E_0 , Energy for a cation at M1; E_s , energy for a cation at saddle point; E , 'activation energy' (kcal mol^{-1}); k , force constant ($\text{kcal mol}^{-1} \text{ Å}^{-2}$); $\tilde{\nu}$, wavenumber (cm^{-1}); $D_{1,200}$, diffusion coefficient at 1,200 °C ($\times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$); values in parentheses are experimental values from ref. 3.

* Self-diffusion.

† From ref. 15.

Fig. 1 Energy map for shifting the coordinate of Mg ion out of the M1 site of forsterite (α - Mg_2SiO_4). Numbers on curves denote energies in kcal mol^{-1} . Numbers in parentheses denote the coordinates at the corners in angstrom unit from the origin. O, M1 site (Mg ion) on the centre of symmetry at the origin (0, 0, 0). $\times$, Saddle point with coordinate (0.5, 0.9, -1.5). There is a mirror plane perpendicular to the z -axis through this point. Cell parameters are: a , 4.756; b , 10.207; c , 5.98 Å (ref. 8).



of the energy barrier to be the 'activation energy' of the atomic diffusion for the octahedral cation.

We looked for a minimum energy route for the Mg ion in the forsterite structure by the following procedure. We calculated the potential energy by shifting the coordinate of the Mg ion in all directions around the octahedral cation sites and drew energy maps. Although for the cation motion a single Mg atom moved in this model, other Mg atoms were moved cooperatively because of the restriction that the symmetry of the olivine structure be maintained as the Mg was displaced. We also reduced the symmetry to P1 in the calculation, but only minor differences of the activation energy have been noticed; all other ions were not allowed to relax. The maps for both the M1 and M2 sites of the Mg ion in forsterite were made and we found that the following route gives the minimum energy barrier. The Mg ion in the M1 site at the origin (0, 0, 0) goes through the saddle point at the fractional coordinate (0.11, 0.09, -0.25) of the cell and it reaches the neighbouring M1 site at (0, 0, -1/2) (Fig. 1). In our model, any other routes such as M1-M2 route^{13,14} gave higher energy barriers than the above route. Therefore, the route for which the Mg ion in the M1 site follows a zig-zag course along the c direction is the minimum-energy route. This result explains the experimental result³ that the Mg ion is the most mobile towards the c direction.

Because the cation in the M1 site is more mobile than that in the M2 site, the Mg ion in the M1 site in forsterite was substituted for a transition metal and we calculated the potential energy of equation (1) by shifting the coordinate of the transition metal in the M1 site. The cell parameters were not changed when the transition metal was substituted. In a similar procedure to the Mg ion in the M1 site in forsterite, the transition metal in the M1 site was moved in a zig-zag way along the c direction. The position of the lowest energy barrier (saddle point) is almost the same for the transition metals and the Mg ion. From the energy map of each transition metal, we estimated the 'activation energy' (Table 2).

The 'frequency term' of the atomic diffusion of the M1 atom was estimated as follows. The shape of the curve of the energy map around the M1 site in the direction of the atomic diffusion was approximated by a curve of second degree on the basis of the simple harmonic oscillation theory and the force constant (k) was obtained. We calculated the frequency of the harmonic oscillator by using the force constant and mass of the cation in the M1 site (Table 2).

The atomic diffusion coefficients (D) calculated from the activation energy, and the frequency term thus obtained are compared with those determined experimentally³ in Table 2. Our results obtained by the simple ionic model can account for the trend of D of the transition metals in forsterite. For example, D of the Fe ion in forsterite is the largest among the transition metals measured.

Table 2 shows that the calculated activation energies do not agree well with the experimental values. Therefore, agreement between the experimental and theoretical diffusion coefficient at 1,200 °C may be fortuitous and deviations in the D values at all other temperatures may be significant. However, the observed values of the activation energy involves noticeable experimental error³. The temperature where the diffusion coefficients are measured for all the transition metals is 1,200 °C. At least, our model seems to explain the order of the atomic diffusion coefficients.

The value D of the Mg ion in forsterite in Table 2 deviates considerably from the experimental value³. This may be because the diffusion process of the Mg ion in forsterite is 'self'-diffusion, and is thus different from that of the other transition metals.

Although the atomic diffusion processes in minerals may be controlled by many complicated or possibly unknown factors, a relatively simple classical ionic model such as that proposed here seems to be moderately successful in explaining the order of the atomic diffusion coefficients in the mineral. However, we have to admit that the agreement between the experimental and calculated values does not guarantee the generality and validity of the repulsive parameter and of the model for the diffusion process used in this study when applied to other structures. To make the models more realistic, the energy of vacancy formation will be relevant, and other problems such as vacancy concentrations, oxygen relaxation, and cooperative processes should be considered. Some of these may not be handled by a static simulation. Although some limitations of the WMIN approach may be encountered in such simulations, our approach may be applicable to estimating the diffusion coefficients of cations which play an important part in geological phenomena but are difficult to obtain experimentally. Our method may also be applied to estimating the order of the atomic diffusion of Ca in pyroxene.

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O-H stretching vibrations in ice clathrate

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In H-bonded materials, the frequency of intramolecular vibrations is considered to depend only on the strength of the H-bond, or equivalently, the distance between the H-bonded molecular pairs. Therefore, in vibrational spectroscopic studies of the molecular structure of condensed phases with O-H...O bonds, the frequency of O-H stretching vibrations, ν , is generally taken as being directly proportional to the average distance, R_{O-O} , between the nearest oxygen atoms. Thus $(\partial\nu/\partial R_{O-O})$ obtained from a study of various polymorphs of ice (R_{O-O} range, 2.75–2.83 Å) has been used as a scaling factor to suggest: (1) a continuous random network structure of amorphous solid and liquid water^{1,2}, and (2) the possibility of transformation of ice VII to a structure with centrosymmetric hydrogen bonds³. While such scaling of R_{O-O} from changes in ν may seem a good approximation, studies of hexagonal ice and ice clathrate suggest that factors other than the intermolecular distances affect the frequency of O-H stretching vibrations. A study of the Raman spectrum of ice clathrate, reported here, shows that in condensed phases where the O-H...O bonds are nonlinear and/or the intermolecular distances are distributed over a range (as in water, various amorphous and crystalline forms of ice, ice-like structures, alcohols, carboxylic acids and aqueous solutions), a treatment based on such scaling is an oversimplification; the geometry of the H-bond affects the frequency more than the strength of the H-bond.

Water molecules in ice clathrates are linked together by H-bonds in the closest packing of polyhedral cage-like structures (host lattice), which can accommodate a variety of molecules (guests). In the two principal types of ice clathrates, the structure of the host lattice is determined mainly by the size of the guest molecule inside the cage. Ice clathrate of type II, studied here, is formed by guest molecules of size 5.6–6.6 Å, and its structure has the larger cages between the two types. Its unit cell⁴, illustrated in Fig. 1, contains 136 water molecules in a framework of 16 dodecahedra and eight hexakaidecahedra⁵. There are seven O-O-O angles in the range 105.73–119.87° and four O-O distances, 2.767, 2.776, 2.796 and 2.812 Å. The average deviation of the O-O-O angles from the tetrahedral value (109.47) is 3.0° and the weighted average O-O distance is 2.790 Å (ref. 5). The host lattice has a density of 0.7845 g cm⁻³.

Tetrahydrofuran ice clathrate (C₄H₈O · 17H₂O) was prepared by freezing at 275 K a solution of C₄H₈O in D₂O or H₂O of the required molar concentration. A cylindrical steel vessel with three sapphire windows was used as the high-pressure optical cell. The cell was filled with silicone oil which acted as a pressure transmitting fluid. The incident light was either 5,145 or 4,880 Å wavelength obtained from a Spectra-physics argon-ion laser. The light scattered by a cylindrical sample of the clathrate kept inside the cell was collected at 90° to the direction of the laser beam and its intensity was measured with a calibrated Jarrel-Ash model 25-400 Raman spectrometer using a spectral slit width of 7–8 cm⁻¹.

Typical unpolarized Raman spectra of the O-D stretching region of D₂O ice clathrate are shown in Fig. 2. The features in the spectrum differ from those of hexagonal ice at 255 K in that the two peaks to the high frequency side of the main peak are not resolved in the clathrate; they are well resolved in ice⁶. The spectrum is essentially similar to the one measured near 100 K in our study of the clathrate, except for the width of the

main peak which is much broader in Fig. 2, due mainly to the increase in the distribution of energy states with temperature. The O-D and O-H stretching frequencies in the clathrate are plotted against temperature at 1 bar and against pressure at a constant temperature in Fig. 3. The value of ν , its rate of change with temperature, $(\partial\nu/\partial T)_P$, and with pressure, $(\partial\nu/\partial P)_T$, are given in Table 1. The quantity $(\partial\nu/\partial P)_T$ showed no variation with temperature in the range 270 > T > 250 K.

The rates obtained from Fig. 3 are related to the change in ν with temperature at a constant volume, $(\partial\nu/\partial T)_V$, by

$$(\partial\nu/\partial T)_V = (\partial\nu/\partial T)_P + (\alpha/\beta)(\partial\nu/\partial P)_T$$

where α and β are the isobaric expansivity and isothermal compressibility, respectively. The expansivity of the clathrate is approximately the same as that of hexagonal ice⁷, and the compressibility is not likely to be much different. The ratio of the two quantities, (α/β) , may thus be the same as for ice⁶, that is, 13 ± 1 bar K⁻¹. From these values we calculate $(\partial\nu/\partial T)_V = 0.28$ cm⁻¹ K⁻¹ for O-D and 0.38 cm⁻¹ K⁻¹ for O-H in the clathrate. The corresponding values of these quantities in ice are included in Table 1.

Table 1 shows that ν intrinsically depends on temperature and $\approx 70\%$ increase in ν at 255 K is not caused by a change in R_{O-O} . Thus the generally accepted correlation between ν and R_{O-O} is questionable. This intrinsic dependence on temperature, of course, causes the isobaric rate of change of ν with R_{O-O} , $(\partial\nu/\partial R_{O-O})_P$, in ice to become 3.5 times the isothermal rate, $(\partial\nu/\partial R_{O-O})_T$, (Table 1). A similar difference between the two rates should also exist in the clathrate. The relatively high value of $(\partial\nu/\partial T)_V$ in the clathrate, as in ice⁶, indicates thermally induced bending of O-H...O linkage and the consequent weakening of the H...O bond at a constant R_{O-O} , and not a decoupling of O-D or O-H oscillator, for the relative magnitude of $(\partial\nu/\partial T)_V$ remains unaltered when O-D or O-H in ice are isotopically decoupled⁸. The Grüneisen constant,

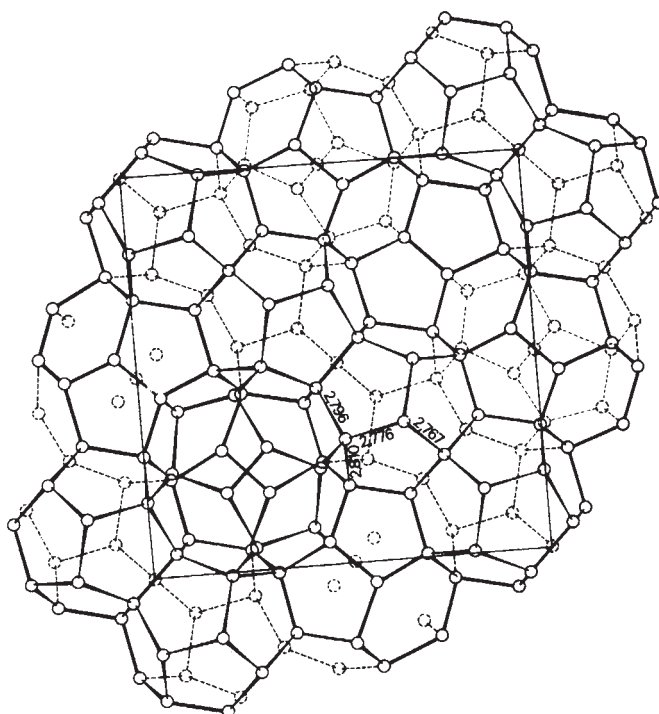


Fig. 1 The crystal structure of ice clathrate of type II in space group Fd3m. The unit cell is marked by the border. $a = 17.31$ Å. Circles represent oxygen atoms. The arrangement of hydrogen atoms is disordered, but they lie close to an oxygen atom along the line joining the two oxygen atoms. The three types of statistically distinguishable water molecules and the distances are indicated.

$-(\partial \ln \nu / \partial \ln V)$, in the clathrate is slightly higher than in ice (Table 1).

The frequency of O-H stretching vibrations depends on the strength of the H-bond, but any influence on the displacement of the H-atom by the directionality of the H-bond should also affect the frequency. The average H-bond in the clathrate structure (Fig. 1) is longer and consequently weaker than in hexagonal ice for two reasons: (1) the average R_{O-O} in the clathrate is 0.03 Å greater than ice (2.76 Å at 255 K) and (2) the O-H...O linkage in the clathrate is nonlinear, as the O-O-O angles in it (range, 105.7°–119.9°) differ considerably from the H-O-H angle (104.5°) of the free water molecule. If ν were to depend only on the strength of H-bond through R_{O-O} , or through the bending of O-H...O linkage (as occurs on heating at a constant R_{O-O}), then ν in the clathrate should be higher than in ice by at least 30 cm⁻¹ for O-D and 45 cm⁻¹ for O-H, as calculated for $\partial R_{O-O} = 0.03$ Å using $(\partial \nu / \partial R_{O-O})_T$ in Table 1. The nonlinearity of the O-H...O linkage and the presence of tetrahydrofuran molecules inside the lattice should further raise these values. The measured ν in the clathrate (Table 1) is higher than in ice by 3 cm⁻¹ for O-D and 4 cm⁻¹ for O-H, nearly one-tenth of the expected increase. Thus in a structure where the strength of the H-bond is lessened for reasons (1) and (2), there is a compensating decrease in ν .

In a condensed phase with nonlinear O-H...O linkage, the potential energy of the O-H stretching vibrations is given by the sum of terms due to change in the: (1) O-H bond length, and (2) O-H...O angle. Here the two changes are not independent of each other, for a periodic displacement of the H atom also causes a periodic change in the O-H...O angle. The displacement of the H-atom is also not colinear with the covalent O-H bond, but deviates in the direction of the H-bond of the O-H...O linkage (a similar situation would exist in materials where H-bonding involves atoms other than oxygen). The compensating decrease in ν , therefore, must arise from the strong coupling between the O-H stretching and O-H...O bending

Table 1 O-D and O-H stretching frequencies in ice clathrate type II and hexagonal ice at 255 K

Property	Ice clathrate		Hexagonal ice	
	O-D	O-H	O-D	O-H
ν (cm ⁻¹)	2,326	3,144	2,323	3,140
$(\partial \nu / \partial T)_P$ (cm ⁻¹ K ⁻¹)	0.42	0.58	0.42	0.63
$(\partial \nu / \partial P)_T$ (cm ⁻¹ kbar ⁻¹)	-10.8	-15.6	-8.9	-13.7
$(\partial \nu / \partial T)_V$ (cm ⁻¹ K ⁻¹)	0.28	0.38	0.31	0.45
$(\partial \nu / \partial R_{O-O})_P$ (cm ⁻¹ Å ⁻¹)			3,620	5,430
$(\partial \nu / \partial R_{O-O})_T$ (cm ⁻¹ Å ⁻¹)			990	1,520
$(\partial \ln \nu / \partial \ln V)$	-0.39	-0.41	-0.32	-0.36

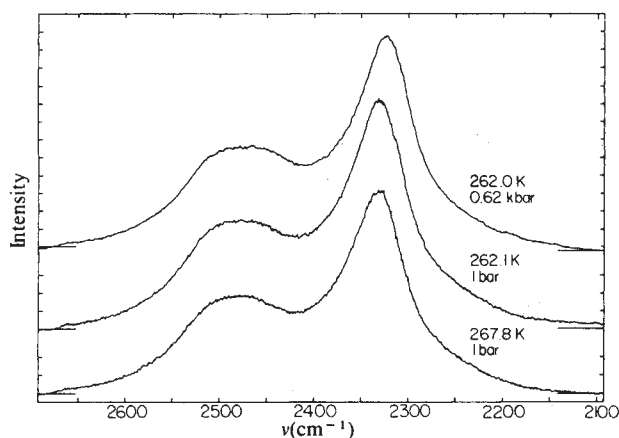


Fig. 2 Raman spectrum of tetrahydrofuran ice clathrate in the O-D stretching region, showing the effect of temperature and pressure on the frequency of the main peak due to the O-D stretching vibrations.

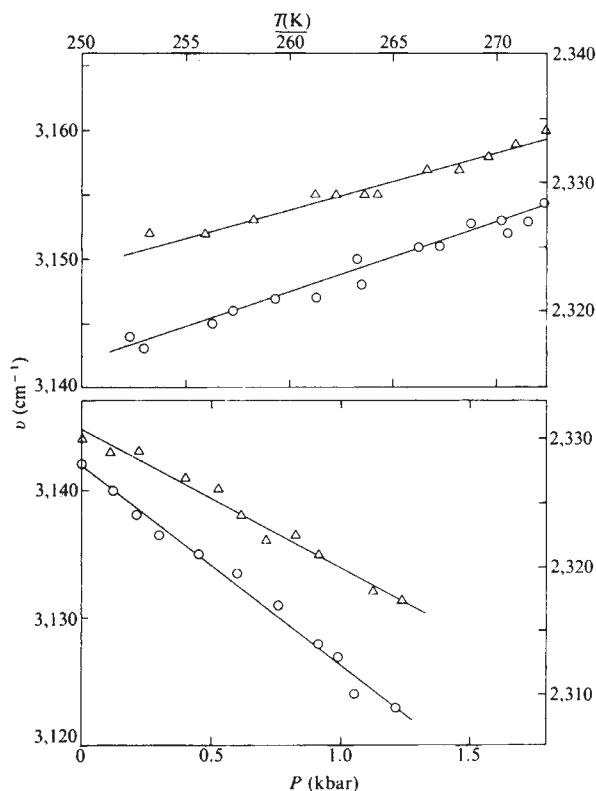


Fig. 3 The frequency of O-D (scale on the left) and O-H stretching vibrations in tetrahydrofuran ice clathrate at 1 bar plotted against temperature (a) and against pressure (b) at 255 K for H₂O and at 262 K for D₂O ice clathrate. Δ , O-D stretching frequency; $\odot$, O-H stretching frequency.

motions, and from the deviation in the displacement of the H-atom from linearity with the covalent O-H bond—a situation that exists in the clathrate but not in ice. Since the bending of the H-bond occurs when both the intermolecular distances and angles are distributed over a wide range of values, we suggest that the intramolecular vibrations is affected more by the arrangement of the neighbouring molecules than by the intermolecular distance.

Bent H-bonds are a common occurrence in carboxylic acids, alcohols, the various condensed phases of water, aqueous solutions and solutions in organic bases, and, as N-H...O linkage, in amides and amino acids. The current approach^{1,2} to understanding the structure of above materials seems inadequate, for our discussion shows that ν increases with temperature even when the intermolecular distance, or volume is held constant, and that the value of the coefficient, $\partial \nu / \partial R_{O-O}$ in Table 1 depends on the method, compression or cooling, used to change R_{O-O} . A further conclusion concerns the effect of the geometry of the H-bonds on the intramolecular vibrations. When a free water molecule becomes H-bonded, the frequency of its O-H stretching vibrations decreases from 3,652 cm⁻¹ in the vapour to ~3,140 cm⁻¹ in ice. This decrease is considered to be more, the more the strength of the H-bond in the condensed phase. Our results suggest that the decrease is greater when bent H-bonds are formed, that is, the arrangement of molecules in the condensed phase has a considerable effect on the frequency.

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Vorticity balance for mid-ocean mesoscale eddies at an abyssal depth

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Current fluctuations in mid-ocean are dominated by mesoscale eddies, which have typical time scales of weeks to months and horizontal scales of tens to hundreds of kilometres. Such mid-ocean eddies may have an important part in the general ocean circulation, and intensive efforts have been made to observe current and density fluctuations in the world's oceans¹⁻⁴. Data sets describing eddy field in a selected region such as the Mode¹ and Polymode³ regions have been obtained in the western North Atlantic. The local dynamics of eddy motions, however, have seldom been discussed on the basis of field measurement data. This report examines the vorticity balance for mesoscale eddies as observed from current meter data. Currents at an abyssal depth were measured at five mooring stations in a cross pattern in the mid-ocean of the western North Pacific. Relative vorticity is estimated from horizontal derivatives of 10-day mean velocities. Its local time change is accounted for by the advection of planetary vorticity within the estimated error. The vorticity balance is primarily linear and barotropic for mesoscale eddy motions at this depth.

Theoretically, the eddy field should be horizontally non-divergent to the lowest order, and potential vorticity should be conserved along a particle path to the first order. Because the Rossby number is small, all properties may be expanded in that number⁵. To the lowest order in the Rossby number, the velocity field is horizontally non-divergent and in geostrophic balance. To the first order, potential vorticity should be conserved along a horizontal particle path; the local time change of relative vorticity should be balanced by the sum of three terms—the advection of planetary vorticity, the advection of relative vorticity, and vortex stretching.

The present observation site is in the mid-ocean of the western North Pacific and is located over fairly flat bottom topography. The site is centred at 30°00' N, 146°40' E (see Fig. 1a). Climatologically, it is ~500 km south of the Kuroshio Extension, and is located on the indistinct southern boundary of the broad, west-southwestward flowing Kuroshio Counter-current at a shallow depth⁶ and in a sluggish northward flow at an abyssal depth⁷. The site is ~400 km away from the Izu-Ogasawara Ridge, there are no pronounced features in bottom topography, and the depth of the water is ~6,200 m.

The current meter data were obtained at 5,000 m depth at five stations in a cross pattern for a common measurement period of 152 days. Figure 1a shows the mooring array configuration. Zonal and meridional separations of stations are either about 45 or 90 km; irregular configuration came from a primary object of measurement to observe fluctuating currents in two different scales and estimate their horizontal extent. Aanderaa RCM-5 current meters were deployed at nominal depths of 4,000 and 5,000 m in early October 1978 and recovered successfully in mid-March 1979. Good quality records were obtained over 152 days from all current meters except at the 4,000 m depth at station TA.

The data sampled at intervals of 30 min are averaged over 1 day to eliminate diurnal and semidiurnal fluctuations. Figure 1b shows daily mean velocities at 5,000 m depth after subtraction of record length averages. They are dominated by low-frequency fluctuations having typical time scales of several tens of days. Daily mean velocities at 4,000 m depth (not shown

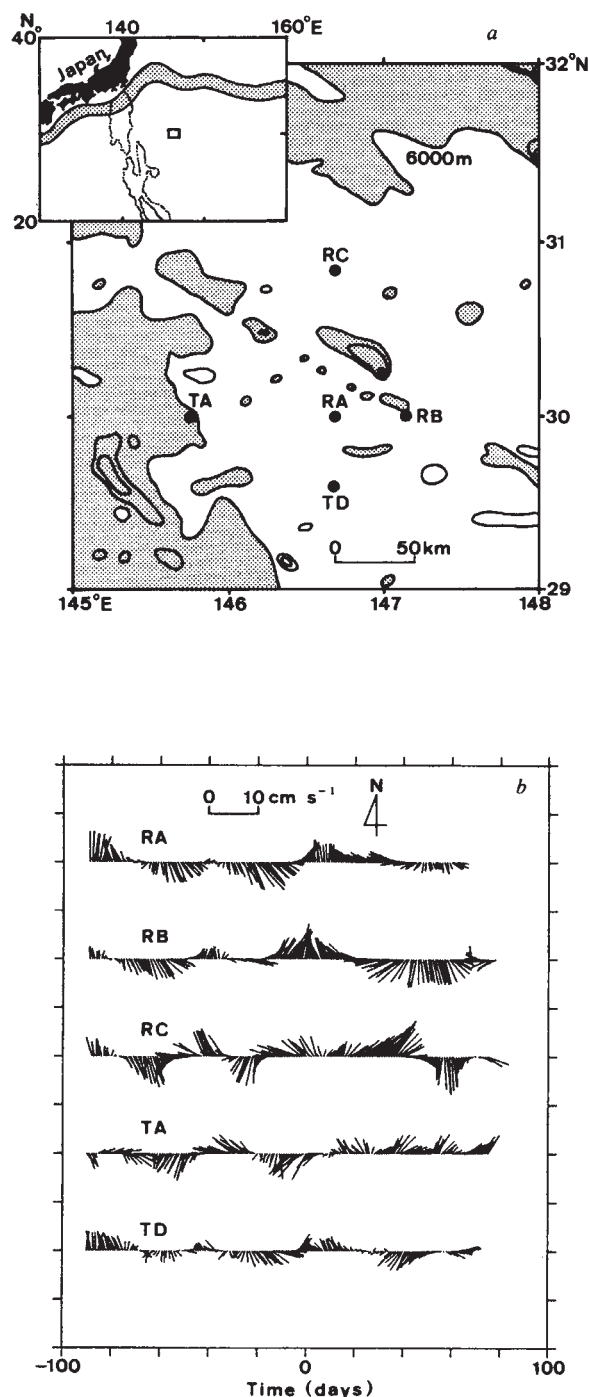


Fig. 1 *a*, Mooring array configuration with a sketch of bottom topography. Shaded areas indicate water depths of <6,000 m. Contour intervals are 500 m. The rectangle within the small-scale physiographic inset illustrates the location of the present study area relative to both the Kuroshio system, whose approximate climatological mean path⁶ is shown by shading, and the Izu-Ogasawara Ridge, whose horizontal extent at 3,000 m depth is shown by dotted lines. *b*, Time series of daily mean velocities observed at 5,000 m depth at the five stations shown in *a* from 2 October 1978 to 19 March 1979. Each stick represents a daily current vector (upward north). The abscissa is time in days; its origin is 31 December 1978. Here, velocities averaged over the record length are subtracted from original daily mean velocities. They are fairly small (0.4 to 2.7 cm s^{-1}) except at station TA, where the averaged velocity is 6.1 cm s^{-1} to the south-southeast. This remarkably large velocity at station TA is due to an intense, almost steady current with bottom intensification¹², whose dynamics seem to differ from the present eddy dynamics.

here) resemble quite well those at 5,000 m depth; thus, the eddy motions are almost uniform vertically at an abyssal depth. The kinetic energy per unit mass for low-frequency motions averaged over all stations is estimated at both depths to be $7.3 \text{ cm}^2 \text{ s}^{-2}$, which is almost the same as a value of that energy at station RB estimated from a subsequent measurement for ~ 3 yr (ref. 8).

Relative vorticity is estimated directly from horizontal derivatives of 10-day mean velocities. Horizontal divergence and the vertical component of relative vorticity at station RA are estimated in a straightforward manner from horizontal derivatives of observed horizontal velocities; here, velocities at middle points between stations RA and RC, and between stations RA and TA are defined by averages of velocities at respective two stations. Typical horizontal scales of fluctuations are estimated to be 70–90 km from the lag of first zero-crossing of transverse correlation function. Therefore, the spacing of stations is not coarse compared with the eddy scales. Figure 2a shows 10-day mean estimates of horizontal divergence and relative vorticity. Because dynamically, horizontal divergence should be zero to the lowest order, the standard deviation of estimated horizontal divergence ($0.18 \times 10^{-6} \text{ s}^{-1}$) can be regarded as the error in estimation of horizontal derivatives. Estimated relative vorticity is large compared with this error; its r.m.s. ($0.57 \times 10^{-6} \text{ s}^{-1}$) is about three times as large as the estimated error.

The local time change of relative vorticity is balanced by the advection of planetary vorticity within the estimated error. The local time change of relative vorticity at station RA is estimated by subtracting the 10-day mean values of vorticity and dividing by 10 days. The advection of planetary vorticity is estimated by multiplying the northward component of 10-day mean velocity at station RA by the meridional variation β ($2.0 \times 10^{-13} \text{ cm}^{-1} \text{ s}^{-1}$ in this latitude) of the Coriolis parameter. The results are shown in Fig. 2b. These two properties have opposite phases and almost equal magnitudes. They have a correlation coefficient of -0.83 , which is significantly different from zero at a 95% confidence level. The r.m.s. of the local change of relative vorticity ($0.51 \times 10^{-12} \text{ s}^{-2}$) is almost equal to that of the advection of planetary vorticity ($0.50 \times 10^{-12} \text{ s}^{-2}$). The standard deviation of the sum of these two properties ($0.29 \times 10^{-12} \text{ s}^{-2}$) closely agrees with the estimated error of that sum ($0.30 \times 10^{-12} \text{ s}^{-2}$).

The vorticity balance is primarily linear and barotropic for mesoscale eddy motions at this depth. The remaining two terms in the vorticity equation, namely, the advection of relative vorticity and vortex stretching, cannot be estimated accurately from the present data; in particular, the latter comes from weak horizontal divergence whose estimate cannot be accurately determined from horizontal derivatives of observed velocities as suggested above. These two terms, however, are not likely to be important in the vorticity balance, because the local change of relative vorticity is almost accounted for by the advection of planetary vorticity.

The result is in striking contrast to previous findings^{9,10} that the advection of relative vorticity as well as vortex stretching are likely to be important in the vorticity balance for mesoscale eddy motions at shallow and mid depths. A relatively similar result has been obtained, however, from a recent examination¹¹ of float trajectories following a topographic-planetary Rossby wave; the trajectories are accounted for by the conservation of barotropic potential vorticity along a particle path, although it is not known whether the dynamics are nearly linear.

The present result indicates that mesoscale eddy motions at an abyssal depth over flat bottom topography in mid-ocean apart from intense current zones tend to be characterized mostly by linear, barotropic Rossby waves, for which the local change of relative vorticity is balanced by the advection of planetary

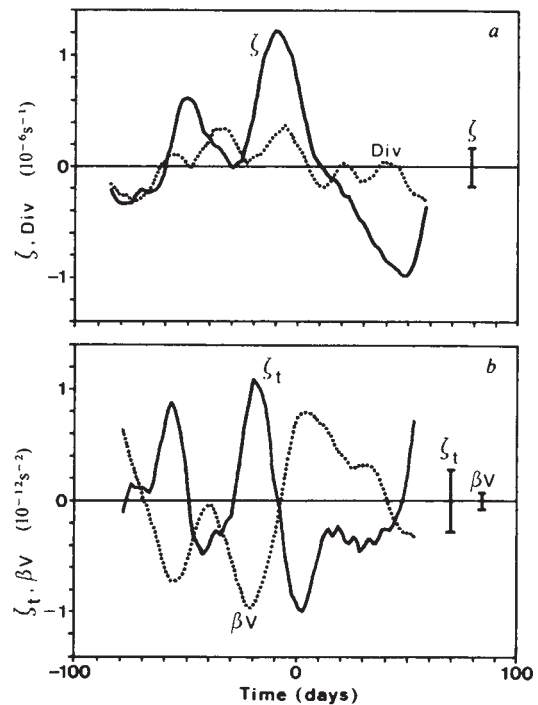


Fig. 2 *a*, Time series of relative vorticity (ζ , solid line) and horizontal divergence (Div, dotted line) estimated directly from horizontal derivatives of velocities shown in Fig. 1. The vertical bar on the right indicates the estimated error of relative vorticity (see text). *b*, Time series of the local time change of relative vorticity (ζ_t , solid line) and the advection of planetary vorticity (βV , dotted line). Error bars for these estimates are shown on the right. The error of the local change of relative vorticity is regarded as $\sqrt{2}/\Delta t$ multiplied by the estimated error of relative vorticity, where Δt is the time interval of differentiation (10 days); it is estimated to be $0.29 \times 10^{-12} \text{ s}^{-2}$. The measurement error of meridional velocity is regarded as equal to the standard deviation of difference in the 10-day mean velocities between depths of 4,000 and 5,000 m, because the eddy motions are almost uniform vertically at an abyssal depth as is noted in the text and the 10-day means should be the same for measurements at these two depths; that is 0.45 cm s^{-1} . Therefore the error of the advection of planetary vorticity is estimated to be $0.08 \times 10^{-12} \text{ s}^{-2}$.

vorticity, although the result does not forbid weak non-linearity of importance to deformation and interaction of these waves.

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Intralayer transitions in phyllosilicates of Martinsburg shale

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Phyllosilicates that occur in shales and slates show complex features as generally detected by methods such as X-ray diffraction. Diffraction patterns commonly display features such as broadened and asymmetric peaks which in many cases have been shown to reflect random interlayering of more than one kind of phyllosilicate¹; for example, illite-smectite and vermiculite-chlorite. Ordered interlayering of more than one phase has also been frequently observed. However, powder diffraction data generally provide insight only into the differences between units of many layers and then they only yield average values. The possibility of variations within individual layers can be investigated using transmission electron microscopy (TEM) and analytical electron microscopy (AEM). We report here on the occurrence of transitions within individual phyllosilicate layers from one trioctahedral structure to another (ferroan lizardite to chlorite) and from trioctahedral to dioctahedral (illite) layers. These variations in structure introduce a new range of complexities into those non-equilibrium phyllosilicate structures which occur in low-temperature sedimentary environments.

The samples studied represent a range between shale and slate and are from the Martinsburg Formation at Lehigh Gap, Pennsylvania. These materials have been studied by various techniques and the phyllosilicates have been shown to consist primarily of a white mica which is transitional from illite (in shale) towards ordered 2M mica (in slate), and chlorite^{2,3}.

Samples (made available by Robert Wintsch of Indiana University) were first prepared as thin sections oriented normal to both bedding and cleavage (so that structure layers of clay are normal to the section). Following optical investigations, selected portions were ion-thinned. They were studied using a JEOL JEM-100CX electron microscope which has an approximate X-ray spatial resolution of 300 Å when operated as an analytical electron microscope and a TEM lattice image resolution of 3.4 Å. Phyllosilicate grains were initially imaged in normal TEM mode. Where appropriate, selected-area electron diffraction patterns were obtained, followed by the recording of 00 l lattice fringe images. In most cases a series of through-focus images was taken to obtain the images that truly reflect the local structure. Fringes were found to reflect the true periodicity in focus conditions for highest contrast images. Areas of the sample so characterized were then subjected to AEM analysis. Energy dispersive X-ray spectra only from thinned areas not subject to atomic number, absorption and fluorescence correction factors were compared with those from well analysed standards, using the methods of Cliff and Lorimer⁴, to obtain semiquantitative analyses. The relevant laboratory procedures and techniques have been described elsewhere^{5,6}. Final characterization of phases was obtained considering collectively, selected-area electron diffraction patterns, lattice fringe images and AEM data. Comparison of the lattice images to calculated images, however, was not carried out, partly because rapid beam damage precluded obtaining the required images.

Most phyllosilicate grains were found to be a dioctahedral phase which is dominantly 1M illite in shale and 2M muscovite in significant amounts in slate, trioctahedral chlorite, or interlayered illite and chlorite; these are described elsewhere^{2,3,7}. Regions of some grains also consist of a phase with 7-Å periodicity and have a composition corresponding to ferroan lizardite, that is, they are trioctahedral phases rich in Mg and Fe

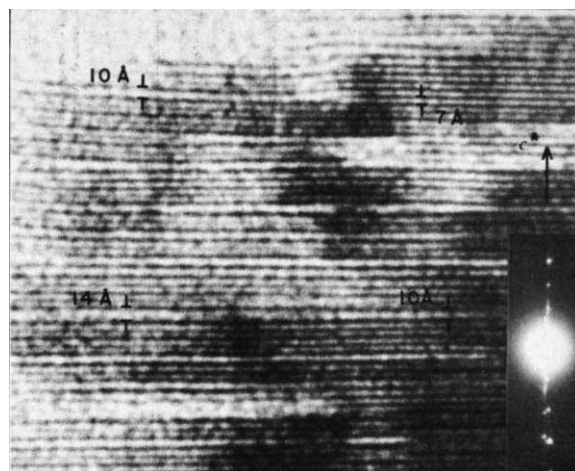


Fig. 1 Lattice fringe image of an area that shows interlayering of 10-Å illite, 14-Å chlorite and 7-Å ferroan lizardite. The labelled layers illustrate transitions from 10-Å illite to 7-Å ferroan lizardite and to 14-Å chlorite within individual layers. The inserted electron diffraction pattern shows (00 l) reflections of 14-Å chlorite streaked along c^* caused in part by random intermixing of 10-Å illite layers. The nonstreaked 10-Å reflections (slightly off angle from the chlorite pattern) are from adjacent illite grains. The specimen is from the transitional sample.

with Fe < Mg. Such grains are found only in samples which do not have a clearly defined slaty cleavage (shales and samples transitional to slates).

Figure 1 is a lattice fringe image of a sequence of phyllosilicate layers which are infrequently encountered but which are the principal subject of this letter. Selected regions are labelled with the appropriate periodicity, showing that there are regions of 14-Å (trioctahedral chlorite), 10-Å (dioctahedral white mica) and 7-Å (trioctahedral ferroan lizardite) structure. These three structures were also identified, in part, through the lattice images of the highest contrast and their characteristic 00 l diffraction patterns (see inset to Fig. 1), which exhibit streaking presumably due to the high degree of disorder seen in the lattice fringe image. Such layers can be seen to randomly alternate in some areas. Such random mixed-layer structures, where trioctahedral and dioctahedral layers are mixed, are unusual. Bailey⁸ has recently reviewed the status of various mixed-layer phyllosilicates, for which there is no firm evidence of dioctahedral-trioctahedral mixed-layering, except, perhaps, for the mineral tosudite. The diffraction pattern of the mixed-layer structures illustrated in Fig. 1 shows that the regions of repeat of a given structure type (14-Å chlorite) are large enough to produce coherent diffraction effects. The randomly interlayered units produce diffuseness in the pattern. In some cases, described elsewhere, regular 1:1, chlorite-mica, mixed-layer sequences occur with a periodicity of 24 Å (ref. 7).

The important feature of Fig. 1 is that there are transitions from one structure type to another along individual layers. These are of two types: (1) transitions from ferroan lizardite (7 Å) to chlorite (14 Å). Sequences of layers can be seen where two individual 7-Å layers (serpentine-type structure) change to a single 14-Å chlorite (talc + brucite-type units) layer. Such a transition involves two phases which have, ideally, the same formula, so their presence is not surprising. (2) Transitions along other layers occur from a 10-Å (illite) phase to either a 7-Å (ferroan lizardite) or 14-Å (chlorite) phase. Such a transformation requires change in both chemistry and structure. The 10-Å phase is Al-rich and dioctahedral, but the 14-Å phase is Fe- and Mg-rich and trioctahedral. In addition to a three layer (talc-like) unit, which is common to the trioctahedral 10-Å phase, the latter has an additional brucite layer. The transition therefore involves a change from a dioctahedral to a trioctahedral unit, with insertion of a brucite layer.

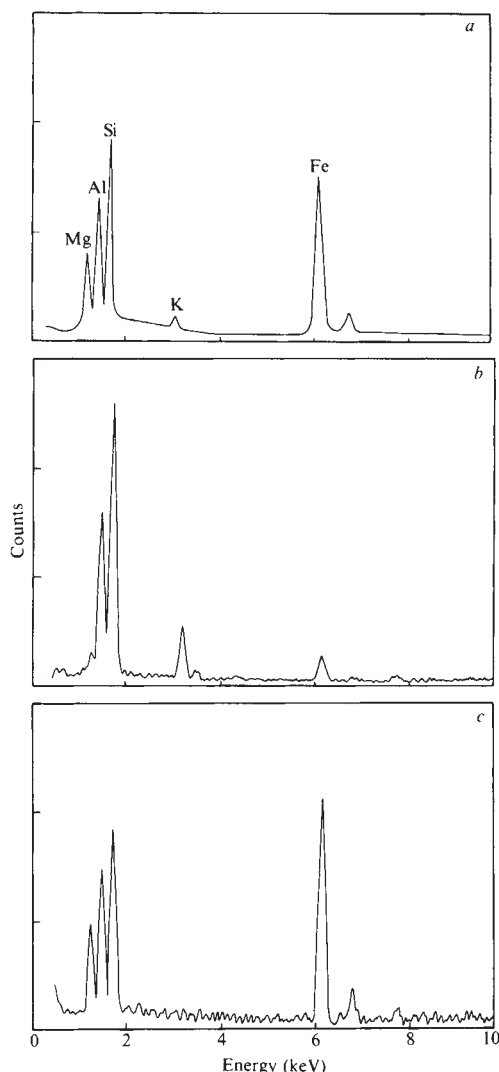


Fig. 2 Energy dispersive X-ray spectra of three different regions: *a*, a type of area shown in Fig. 1 which contains interlayered illite, chlorite and ferroan lizardite (K and high Mg and Fe are characteristic); *b*, illite (high K is characteristic); *c*, chlorite (high Mg and Fe and no K are characteristic). Note that spectrum *a* has characteristics of both spectra *b* and *c*.

Figure 2 shows energy dispersive X-ray spectra from selected areas which are representative of the various structure types. Figure 2*a* is a spectrum from the region for which the kind of lattice fringe image of Fig. 1 was obtained. Figure 2*b,c* shows spectra from other areas of the same sample which yield lattice fringe images (not shown) and other data indicating that they consist of 10- and 14-Å layers, respectively. These two spectra thus serve as standards for the dioctahedral illite (K is characteristic) and trioctahedral chlorite (Mg and Fe are characteristic) layers. The spectrum in Fig. 2*a* displays major amounts of all of these elements, verifying that the 10- and 14-Å layers in Fig. 1 are actually dioctahedral illite (as opposed to trioctahedral 10-Å phases) and trioctahedral chlorite, respectively. If the illite component is subtracted from the polyphase spectrum (Fig. 2*a*), the remaining spectrum must correspond to the 14- and 7-Å layers. Because this composition is nearly identical to that of chlorite, the chlorite (14 Å) and ferroan lizardite (7 Å) must be nearly identical in composition. This confirms the chemical nature of the intralayer and interlayer transitions as described above.

Finally, we note that the intralayer transitions described above are found only where complex interlayered sequences also occur. In addition, they are found only in shales and in samples which are transitional to slate. They clearly represent

non-equilibrium conditions such that given additional time, or increased temperature, or subjected to some other variable which increases reaction rates, they would be caused to anneal away, with the production of stable chlorite or mica. It is just such relatively stable structures which are observed in the slates. Such intralayer transitions may occur quite commonly, however, and their presence adds an additional level of complexity to those phyllosilicate structures which are characteristic of low-temperature, sedimentary diagenesis where chemical equilibrium is difficult to attain.

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Primitive nephelinitic volcanism associated with rifting and uplift in the Canadian Arctic

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Considerable interest has arisen recently in the nature of plate tectonic events in Arctic Canada and northwestern Greenland during Cretaceous to Recent times¹. Relatively little is known, however, concerning contemporaneous volcanism, and detailed studies have only been made of the basalts related to the Baffin Bay spreading centre^{2,3}, and the Kap Washington peralkaline volcanics associated with regional doming and rifting of northern Greenland during the late Cretaceous⁴. We show here that the Freemans Cove volcanic suite forms a major petrological province of Eocene saturated to undersaturated volcanism in the central Canadian Arctic. This occurrence of basalts, nephelinites and phonolites is an example of intraplate magmatism of the type associated with continental rifting. Emplacement of the rocks has been controlled by graben-like faults formed during the Boreal rifting and Eurekan deformation of the Arctic plate. Magmatism was initiated when extensional deformation was transformed by pre-existing resistant structures into a region of compression and uplift, causing partial melting of metasomatized mantle. Primary nephelinite magmas unusually rich in Ba, Sr, La form a major component of the magmatism.

The Freemans Cove volcanic suite occupies a 40×20 km area of southeastern Bathurst Island (98°10' W; 75°5' N). Sills, dykes and small plugs are intrusive into the Lower Devonian Bathurst Island Formation, the Middle Devonian Disappointment Bay Formation, and a down-faulted block of Cretaceous Eureka Sound Formation⁵.

Igneous activity is confined to the southern portions of the south-east Bathurst Fault Zone⁵ an approximately north-south trending system of normal faults. The igneous rocks consist of a suite of tholeiitic, transitional and alkaline basalts, basanites, olivine nephelinites, olivine melilite nephelinites, phonolites and phonolitic trachytes. Representative compositions of the major varieties are given in Tables 1 and 2. (Samples prefixed N and BI are from the 1973 and 1976 expeditions to Bathurst Island respectively. Compositions presented are of individual samples.)

The magmatism appears to be bimodal in character. Rocks of the basanite-nephelinite-phonolite association are predominant and occur as dykes and plugs. Basaltic rocks are confined to agglomeratic vents and sills.

The rocks of the province are characterized by an enrichment in incompatible elements. Figure 1 illustrates rare-earth element (REE) distribution patterns for the major magma types. La/Yb ratios of mafic rocks increase with increasing under-saturation, for example, 14–31 (basalts), 22–30 (basanites/basanitoids), 30–76 (nephelinites). Similar relative light (L) REE enrichments are observed in other alkaline volcanics and it has been postulated that the mantle sources for such volcanics have been metasomatized and enriched in LREE shortly before the onset of volcanism^{6,7}.

The basalts and basanitoids have mineralogical (high Al-pyroxenes) and geochemical ($Mg/(Mg + Fe) < 65$; $Ni < 200$ p.p.m.) features which indicate that they were formed from evolved liquids which experienced high and low pressure fractionation.

Some nephelinites are remarkable in that they exhibit the characteristics of primary magmas, for example, liquids generated by partial melting of a mantle source without subsequent compositional modification.

Such magmas have corrected $Mg/(Mg + Fe)$ ratios ($Fe^{3+}/Fe^{2+} 0.15$)⁷ of 65–75, and Ni contents > 250 p.p.m. together with high abundances of incompatible elements. Representative examples of Bathurst Island nephelinites which meet these criteria are given in Table 3. Especially striking is the wide variation in incompatible element abundances (U, Th, La, Ce, Ba, Sr) at similar $Mg/(Mg + Fe)$ ratios. Strong positive correlations exist between incompatible elements and La or U and a negative correlation with Ni. These relationships are observed in liquids whose major element composition is effectively buffered at constant MgO/FeO and Al_2O_3/CaO ratios and are best interpreted to imply that the magmas were a sequence of primary partial melts.

Unmodified partial melts of mantle material are very rare. Similar primary nephelinites have also been described^{6,7} from Hawaii (Oahu) and Australia, however, none of the primary magmas found in these regions exhibits the extreme Ba, Sr and LREE enrichment found in the most primitive Freemans Cove nephelinites.

Our preliminary geochemical studies indicate that there is no simple fractional crystallization relationship between any of the Freemans Cove magmas. They may represent different degrees of partial melting of a metasomatized mantle source

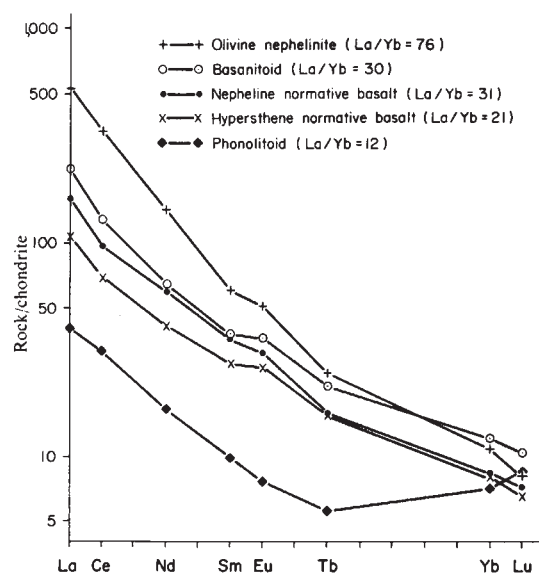


Fig. 1 Chondrite normalized rare-earth element distribution patterns of representative members of the Freemans Cove volcanic suite.

Table 1 Representative basalt and basanitoid compositions

Wt %	NI-6	BI-16	N4-2	BI-63
SiO ₂	46.75	48.65	50.05	44.80
TiO ₂	1.91	1.94	1.83	1.90
Al ₂ O ₃	13.48	15.10	13.38	12.86
Fe ₂ O ₃	4.13	4.38	3.53	2.96
FeO	7.56	5.76	7.12	8.81
MnO	0.15	0.12	0.17	0.19
MgO	9.51	7.09	7.72	11.30
CaO	11.30	10.44	9.11	10.94
Na ₂ O	2.68	2.98	3.06	2.96
K ₂ O	0.70	0.78	0.78	1.12
P ₂ O ₅	0.49	0.34	0.43	0.65
CO ₂	0.10	0.62	0.63	0.10
H ₂ O _T	1.26	1.80	2.06	1.01
Total	100.02	100.00	99.87	99.60

CIPW norms				
or	4.20	4.74	4.75	6.73
ab	19.94	25.93	26.70	10.74
an	23.05	26.24	21.11	18.81
ne	1.68	—	—	7.98
di	24.96	20.19	18.46	25.99
hy	—	5.99	17.70	—
ol	19.12	10.41	4.63	22.32
mt	2.20	1.91	2.04	2.24
il	3.69	3.79	3.58	3.67
ap	1.15	0.81	1.03	1.53

Norms calculated to 100 wt % on a volatile-free basis with $Fe_2O_3/FeO = 0.15$. NI-6, alkali basalt; BI-16, transitional basalt; N4-2, olivine tholeiite; BI-63, basanitoid, or Orthoclase; ab, albite; an, anorthite; ne, nepheline; di, diopside; hy, hypersthene; ol, olivine; mt, magnetite; ap, apatite; il, ilmenite.

Table 2 Representative nephelinite and phonolite compositions

Wt %	BI-4	BI-268	BI-84	N2-6	BI-87
SiO ₂	40.41	39.97	41.90	54.70	56.03
TiO ₂	2.08	1.74	2.04	0.29	0.28
Al ₂ O ₃	14.10	11.15	12.46	21.02	20.66
Fe ₂ O ₃	5.35	4.70	3.89	2.12	2.27
FeO	4.92	6.11	7.44	0.60	0.72
MnO	0.17	0.19	0.19	0.14	0.15
MgO	10.41	14.15	11.55	0.81	0.05
CaO	13.15	15.64	12.99	2.35	1.41
Na ₂ O	4.77	3.00	3.15	6.01	8.80
K ₂ O	1.79	1.32	1.06	5.42	5.57
P ₂ O ₅	0.68	0.87	0.69	0.05	0.06
CO ₂	0.48	ND	0.31	0.72	0.00
H ₂ O _T	1.71	ND	1.94	5.61	3.69
Total	100.12	99.53	99.61	99.84	99.69

CIPW norms					
or	—	—	0.87	34.26	34.29
ab	—	—	—	39.22	36.50
an	11.00	13.18	17.23	12.12	0.44
lc	7.90	6.21	4.37	—	—
ne	23.99	13.93	14.87	8.22	22.25
di	20.14	19.77	35.96	—	0.28
wo	—	—	—	—	2.54
ol	19.40	28.16	18.90	1.51	—
ln	12.37	10.99	—	—	—
he	—	—	—	1.12	0.93
mt	1.26	1.97	2.16	1.16	2.08
il	2.71	3.34	3.99	0.59	0.55
ap	1.24	1.92	1.65	0.12	0.14
c	—	—	—	1.18	—

Norms for analyses 1–3 recalculated to 100% on a volatile-free basis with $Fe_2O_3/FeO = 0.15$. ND, Not determined.

BI-4, olivine melaneophelinite; BI-268, olivine melilite nephelinite; BI-84, olivine nephelinite; N2-6, trachy-phonolite, BI-87, phonolite. lc, Leucite; wo, wollastonite; ln, larnite; he, haematite; c, corundum.

with modification of some compositions by high and/or low-pressure fractionation. We believe that the phonolitoids may be the derivatives of a nephelinitic magma. Certainly contemporaneous consanguineous nephelinite-phonolite magmatism is not unusual for magmatism associated with the East African Rift⁸.

Rb-Sr isochrons for the nephelinite-phonolite magmatism are given in Fig. 2. Isochron 1 using all the data points gives an age of 47.1 ± 4.0 Myr (MSWD = 0.41), isochron 2 which gives a better statistical fit produces an age of 46.9 ± 3.0 Myr (MSWD = 0.0). The ages are statistically equivalent, indicating that the age of the nephelinite-phonolite volcanism is 47 Myr, that is, Lutetian⁹ (middle Eocene).

The age of the basaltic magmatism is as yet unknown. Basaltic sills are interbedded with the Maastrichtian (late Cretaceous) Eureka Sound Formation at the head of Freemans Cove. These are necessarily of post-Maastrichtian age (<65 Myr).

We are not yet able to determine the temporal sequence of the basaltic and nephelinitic volcanism but we are confident that all the igneous activity is post-Maastrichtian and that the alkaline undersaturated magmatism is definitely of Lutetian age.

Volcanic rocks similar to those of Freemans Cove have been described from the Gregory Rift, Kenya¹⁰, the Balcones Province, Texas¹¹, the Heggau and Urach Provinces of the Rhine Graben¹². Similarities with the Newer Volcanics of West Victoria of Australia are also evident⁷. All of these suites are characteristic of intraplate continental magmatism, commonly considered to be associated with rifting and doming¹³. Clearly the Freemans Cove magmatism is therefore not of the type associated with spreading centres.

Kerr¹ has outlined the development of Arctic Canada in terms of opposing, but contemporaneous plate tectonic events and has proposed that during late Cretaceous to mid-Tertiary times the Boreal Rifting episode and the Eureka Deformation were simultaneously fragmenting the continent from opposing sides of the Boothia-Cornwallis Arch. Rift structures in the western Arctic produced in the early Palaeogene by the Boreal

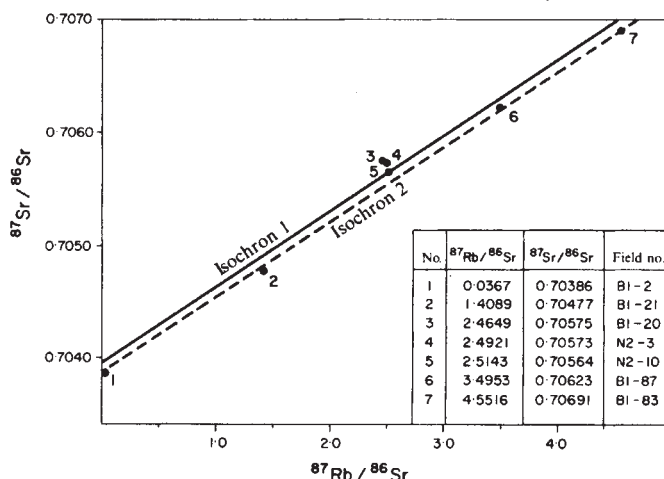


Fig. 2 Rb-Sr isochrons for members of the nephelinite-phonolite suite. Isochron 1, samples 1-7 (MSWD = 0.41), age = 47.1 ± 4.0 Myr, initial ratio = 0.70395 ± 0.00016 . Isochron 2, samples 1, 2, 6, 7 (MSWD = 0.0), age = 46.9 ± 3.0 Myr, initial ratio = 0.70387 ± 0.00012 .

Table 3 Representative compositions of primitive nephelinites, Freemans Cove Volcanics

Wt %	BI-76	BI-13	BI-128	BI-73	BI-98	BI-134	N3-P
SiO ₂	40.90	41.46	41.35	41.50	42.65	39.41	40.65
TiO ₂	1.48	1.85	1.70	1.76	1.80	1.73	1.88
Al ₂ O ₃	11.36	11.49	11.24	11.22	10.42	10.78	10.12
Fe ₂ O ₃	3.43	3.75	3.85	3.54	3.45	4.10	4.17
FeO	7.06	7.32	7.16	7.48	7.24	6.36	6.48
MnO	0.19	0.16	0.20	0.19	0.19	0.18	0.20
MgO	13.96	15.23	14.18	14.22	15.18	13.50	12.55
CaO	14.29	12.85	13.34	13.04	12.03	15.28	14.50
Na ₂ O	3.04	3.15	2.99	3.13	3.13	2.91	2.83
K ₂ O	1.03	1.13	1.31	1.11	0.98	1.03	1.08
P ₂ O ₅	1.29	0.81	0.80	0.79	0.62	0.82	0.67
CO ₂	0.25	0.18	0.27	1.35	1.04	3.04	2.34
H ₂ O _T	1.44	0.72	1.28	0.83	0.88	0.81	2.31
Total	99.72	100.12	99.66	99.81	99.63	99.95	99.78
Sc	27	27	28	27	27	24	27
V	205	230	250	195	210	240	220
Cr	560	650	345	520	850	600	560
Co	66	64	69	73	72	59	64
Ni	328	315	393	359	436	351	324
Cu	90	80	70	75	70	65	45
Rb	21	27	34	24	41	39	23
Sr	2,080	1,040	1,059	944	744	881	708
Y	29	30	—	25	—	—	23
Zr	139	156	—	131	—	—	154
Ba	2,505	1,242	1,314	1,047	798	1,065	912
Ta	5.4	5.4	6.1	4.2	4.8	3.4	4.3
Hf	2.3	3.2	3.1	3.2	4.1	2.9	3.5
Th	20.7	10.4	10.4	10.5	7.7	9.3	5.0
U	4.6	3.0	2.5	2.2	1.7	—	1.5
La	185	122	92	89	64	78	55
Ce	298	207	167	146	107	149	88
Nd	87	51	48	42	39	40	44
Sm	10.9	7.8	8.5	7.8	7.5	7.9	7.2
Eu	3.6	2.8	2.8	2.6	2.4	3.0	2.0
Tb	1.2	1.1	0.8	1.0	0.9	1.1	0.7
Yb	2.3	2.3	2.2	1.8	1.6	2.1	1.3
Lu	0.28	0.29	0.25	0.27	0.22	0.28	0.18

V, Cr, Cu by emission spectrometry; Ni, Cu, Rb, Sr, Y, Zr, Ba by X-ray fluorescence spectrometry; Sc, Co, Ta, Hf, Th, U by instrumental neutron activation analysis; rare earths by radiochemical neutron activation analysis.

Rifting terminated at this resistant block and were transformed into the north-south faults of the south-east Bathurst Island Fault Zone. In the eastern Arctic, the Eurekan Deformation resulted in seafloor spreading with associated tholeiitic magmatism (58 Myr)¹⁴ in the Baffin Bay-Davis Strait area during the Thanetian. Eventually the spreading was impeded by the resistant pre-existing structures and the area of extensional deformation was transformed into an opposed apical region of compression in the Bathurst-Somerset Island area during the middle Eocene to early Miocene. Subsequent Eurekan rifting produced the Lancaster aulacogen and rejuvenated pre-existing Bathurst Island faults.

The middle Eocene Freemans Cove volcanics were emplaced during the initial compression and uplift of Bathurst Island. We propose that the uplift initiated partial melting of the upper mantle, the existing faults of the south-east Bathurst Island Fault Zone allowed the rapid ascent and emplacement of the primitive nephelinites. In an integrated model of magmatism⁷ the less undersaturated melts would represent more extensive partial melts generated after the initial limited melts and during the later stages of uplift.

The geochemistry of the rocks indicates that the partial melts were probably derived from metasomatized mantle. Our hypothesis that their generation was in response to tectonism contradicts Bailey's¹³ hypothesis that uplift is, in fact, caused by metasomatism.

The Freemans Cove volcanics are, therefore, an example of intraplate magmatism of the type associated with continental rifting and are the first recognition of such magmas within Arctic Canada.

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A bicontinuous tetrahedral structure in a liquid-crystalline lipid

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The structure of most lipid-water phases can be visualized as an ordered distribution of two liquid media, water and hydrocarbons, separated by a continuous surface covered by the polar groups of the lipid molecules¹. In the cubic phases in particular, rod-like elements are linked into three-dimensional networks^{1,2}. Two of these phases (space groups Ia3d and Pn3m) contain two such three-dimensional networks mutually inter-

woven and unconnected. Under the constraints of energy minimization³, the interface between the components in certain of these 'porous fluids' may well resemble one of the periodic minimal surface structures of the type described mathematically by Schwarz^{4,5}. A structure of this sort has been proposed for the viscous isotropic (cubic) form of glycerol monooleate (GMO) by Larsson *et al.*⁶ who suggested that the X-ray diagrams of Lindblom *et al.*⁷ indicated a body-centred crystal structure in which lipid bilayers might be arranged as in Schwarz's octahedral surface⁴. We have now found that at high water contents, a primitive cubic lattice better fits the X-ray evidence with the material in the crystal arranged in a tetrahedral way. The lipid appears to form a single bilayer, continuous in three dimensions, separating two continuous inter-linked networks of water. Each of the water networks has the symmetry of the diamond crystal structure and the bilayer lies in the space between them following a surface resembling Schwarz's tetrahedral surface⁴.

The viscous isotropic phase of GMO occurs over the range of temperatures and water content displayed in the phase diagram of Lutton⁸. At room temperature, this phase exists at water contents up to 40% by weight beyond which an excess water phase occurs. Figure 1 shows an X-ray diffraction pattern of GMO at 22 °C in the presence of excess water. In addition to the sharp reflections at low angles shown, a diffuse band at 4.5 Å is found indicating that the hydrocarbon chains of the lipid have a high degree of liquid-like disorder⁹. The crystalline reflections index as 8 of the first 10 orders of a primitive cubic lattice. The innermost reflection observed would then be the 110, which corresponds to the reflection indexed by Lindblom *et al.* as their 222 reflection. However, as no lower orders exist, their assignment is not likely to be correct. For instance, out of their first 28 possible reflections of mono-olein containing 39.87% water there would be at least 20 missing orders to be accounted for, including the five lowest orders.

The *International Tables for X-ray Crystallography*¹⁰ list seven sets of primitive cubic space groups which can be determined by their X-ray patterns. Four of these sets are eliminated by the presence of the 110, 111 and 200 reflections. The absence of the 100 and 210 reflections rules out another two of the seven, leaving only one set containing the two tetrahedral space groups Pn3 and Pn3m. It might be argued that the absence of the 100 and 210 spots could be due to a fortuitous distribution of material within the unit cell. However, diffraction patterns taken at reduced water content show that the unit cell dimensions decrease as the amount of water goes down, while the intensity of these two spots remains zero. It is improbable that such zeros would coincide with the positions of crystal reflections in the different stages of shrinking.

At low resolution, Pn3 and Pn3m are very similar, differing only in the presence of mirror planes in Pn3m, and the use of either leads to similar results. We have chosen to work with Pn3 in the calculation of models. In this space group, the symmetry demands two equivalent sets of 12 asymmetric units situated at positions clustered around points of tetrahedral symmetry. The two tetrahedral clusters are separated by a translation of one half the body diagonal and are related by a centre of symmetry situated half-way between them¹⁰. The tetrahedral symmetry is such that the clusters would become more centro-symmetric if the 12 asymmetric units were moved away from the three-fold axes. If this happened, the unit cell would become more nearly body centred and hence, the 111 reflection would become weak. Since the 111 spot is strong (Fig. 1), it follows that much of the scattering matter in the cell must lie close to the three-fold axis. Hence, we are led to a model consisting of a tetrahedral arrangement of matter lying along the 111 axes.

Models were tested by computing the diffraction amplitudes and phases of various distributions of closely spaced points concentrated around the 111 axes. Since the X-ray data do not extend much beyond 30 Å, the use of more detailed models would not be justified. The observed and calculated amplitudes

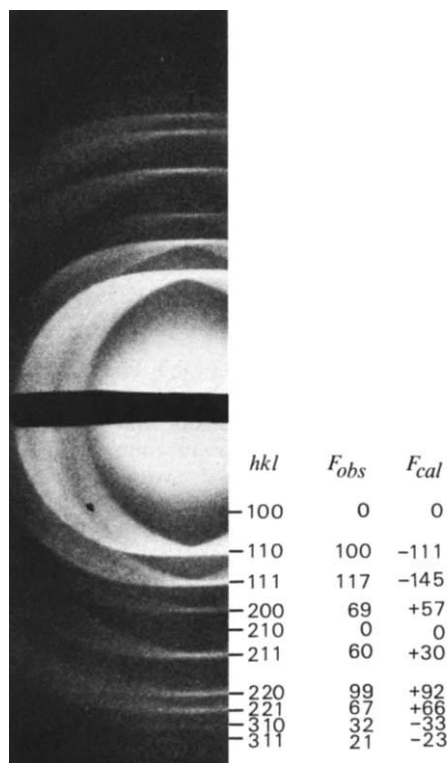


Fig. 1 X-ray diffraction pattern of glycerol mono-oleate at a temperature of 22 °C in the presence of excess water. The length of the cell side is 105 Å. The reflections index on a primitive cubic lattice, space-group Pn3 or Pn3m. The indices are listed beside their corresponding reflections together with the observed and calculated amplitudes. Since the space group is centred, the X-ray phase angles (referred to the centre of symmetry) are either 0° or 180° and are tabulated as + and -, respectively. The photograph was taken with a focusing monochromator camera at a specimen to film distance of 275 mm. Relative intensities were measured by scanning the film on a microdensitometer, and the appropriate geometrical corrections were applied¹⁶.

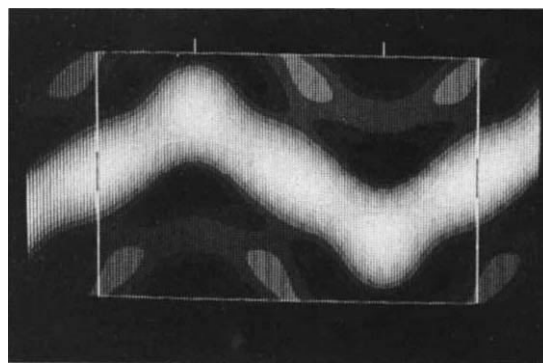


Fig. 2 110 section of an electron density map using the observed amplitudes, with the phases calculated from a model consisting of matter concentrated around tetrahedrally arranged lines parallel to body diagonals of the cubic unit cell. Continuous white channels are arranged as in the carbon-carbon bonds in diamond. Minor peaks are caused by the limited resolution (30 Å) of the map. The unit cell is outlined. The two short vertical lines at the top indicate the position of two-fold axes. Photographed from the screen of a computer video display.

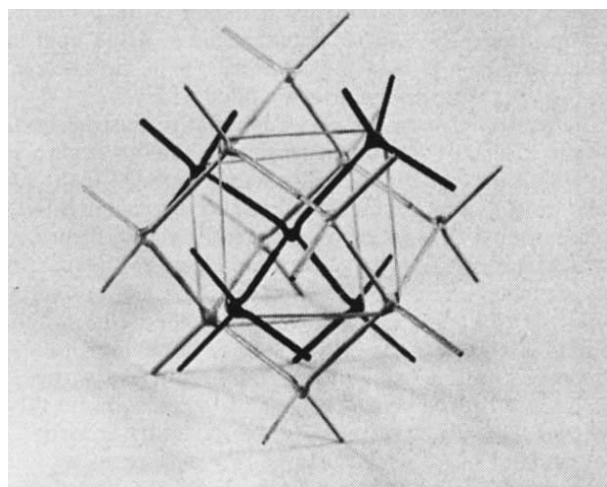


Fig. 3 A wire model with Pn3 symmetry, consisting of two interpenetrating diamond networks, one painted black and one white. Two opposite faces of a cubic unit cell are outlined with rubber bands.

were scaled by making $\sum |F_{obs}|$ equal to $\sum |F_{cal}|$. The model was adjusted by minimizing the 'R-factor'—the commonly used crystallographic figure of merit given by $R = (\sum ||F_{obs}| - |F_{cal}||) / \sum |F_{obs}|$. The best model consisted of continuous rods about 40 Å in diameter, joining together at the tetrahedral points. The final R factor was 0.19. No other type of model was found which gave a satisfactory distribution of amplitudes.

Using the calculated phases of the best model and the observed amplitudes (Fig. 1) we then computed the electron density map whose 110 section is shown in Fig. 2. This section is seen to contain channels of material disposed around axes converging at the points of tetrahedral symmetry. Two other channels radiate from each of these tetrahedral centres and travel out of the plane of the section. A photograph of a wire model encompassing one unit cell of the crystal is shown in Fig. 3. In this model the channels are represented by two networks of rods, each following the symmetry of the interatomic bonds in the crystal structure of diamond, but which together form a primitive cubic lattice having a cell edge length one-half that of the diamond lattice. The two networks interpenetrate but do not make contact with each other. A similar structure consist-

ing of rods oriented tetrahedrally in the space group Pn3m was reported in phosphatidyl ethanolamine^{1,11}. This double diamond lattice is an unusual crystallographic feature first found in the structure of cuprite, Cu₂O, analysed by Bragg in 1916 (see ref. 12). An analogous geometrical arrangement is seen in the structure of turnip yellow mosaic virus¹³ in which two sets of identical particles are related by a bonding pattern of this type.

From our X-ray results alone it is not possible to determine whether the diamond-branched channels contain lipid or water. An answer to this question can, however, be found by considering the amount of lipid in the unit cell. From the phase diagram⁸ this lipid content is seen to be close to 60% by weight at 22 °C in the presence of excess water. Since the density of GMO is <1, the volume of lipid in the cell will be >60%. The volume of the channels can be estimated by using a model made of circular cylinders terminated by hemispherical caps, each having an overall length equal to half the body diagonal of the cell. If the channels contained lipid, their maximum diameter would be limited to twice the length of a GMO molecule. This length can hardly be ≥ 45 Å, the maximum repeat period in the lamellar bilayer phase of mono-olein at 22 °C (ref. 7). Using this

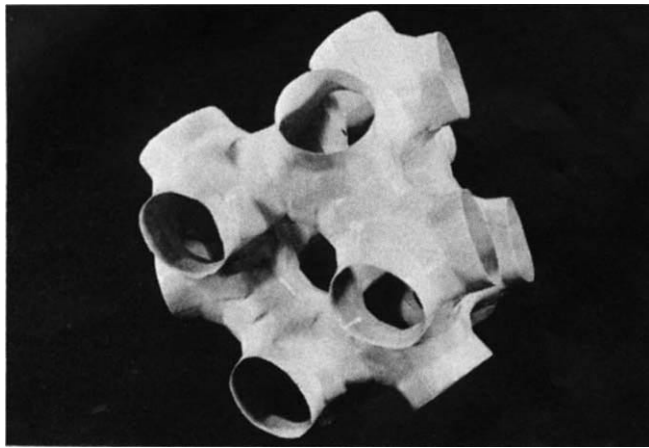


Fig. 4 Model of part of Schwarz's tetrahedral periodic surface, made of strips of paper glued together and painted. This surface would lie within the lipid bilayer which separates the water channels into two equal sub-volumes.

value as an upper bound for the diameter of the cylinders, we find that the calculated maximum volume of the lipid in the model would be 42% of the cell, which is much too low. The conclusion is that the channels are filled with water.

The space between the water labyrinths must be occupied by lipid. This space forms a single, continuous region which contains Schwarz's tetrahedral minimal surface. If the hydrophilic head groups contact the water in the channels with the hydrocarbon tails pointing away from the water, then the lipid forms a bilayer which can be accommodated by the dimensions and symmetry of this space. A model of part of this surface is illustrated in Fig. 4. The surface can be theoretically built up of units, each composed of that surface of minimal area which is bounded by four straight lines connecting the vertices of a regular tetrahedron. The mathematical expression for this surface and illustrations of a model are given by Schwarz⁴, who also explains how the unit can be infinitely repeated to form the complete periodic surface. Each edge of the crystallographic cell passes normally through the centre of a two-fold saddle-surface, while normal to the body diagonals there are three-fold 'monkey saddles'¹⁴.

The viscous isotropic phase of monoglycerides has been found among the digestion products of fat¹⁵. We know of no other naturally occurring example of Schwarz's periodic tetrahedral minimal surface.

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Patterns in vascular land plant diversification

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Statistical analyses indicate that the Phanerozoic history of vascular land plants (tracheophytes) may be interpreted in terms of the successive radiations of four major plant groups, each characterized by a common morphological and/or reproductive grade. Following initial invasion of the land, the diversification of each group coincides with a decline in species numbers of the previously dominant group. Analyses indicate that each group is initially characterized by a high rate of appearance of new species but short species durations. However, within a group through time, new species appear less frequently but species durations increase. Parallels between the diversification patterns observed for tracheophytes and those previously noted for marine invertebrates¹ suggest there are generalized patterns in the evolution of higher taxa.

Studies of the taxonomic diversity of Phanerozoic invertebrates have shown a pattern of changes in diversity through time, and that this pattern reflects an evolutionary phenomenon strong enough to overcome the presumed biases of the fossil record¹. Q-mode factor analyses of marine metazoa further indicate that the overall pattern can be explained in terms of the expansion and subsequent contraction of three sequential, discrete 'evolutionary faunas'². From these studies, quantitative estimates of the rates and magnitudes of major biological radiations and extinctions have been obtained for marine organisms. Palaeobotanists have also been aware of similar patterns in the Phanerozoic history of tracheophytes. Eras of geological time have been identified palaeobotanically as the Palaeophytic, Mesophytic and Cenophytic, corresponding to those periods dominated by the pteridophytes, gymnosperms and angiosperms, respectively³. Our quantitative studies of the patterns of diversification of vascular plants indicate that the fossil record of tracheophytes is as amenable to analysis as that of marine invertebrates^{4–6} and offer the opportunity to characterize major patterns in tracheophyte evolution. Because of the fundamental differences in the biology of invertebrates and vascular plants, as well as differences in the habitats in which they evolved, comparisons between the patterns of diversification for these two groups could provide an opportunity to evaluate the possible existence of unifying patterns in the evolution of life.

Our analysis at the species level (Fig. 1) indicates that the overall pattern of terrestrial plant diversification can be separated into four distinct evolutionary phases: (1) a Silurian–mid-Devonian proliferation of early vascular plants that are characterized by a simple and presumably primitive morphology (Fig. 1a); (2) a subsequent late Devonian–Carboniferous radiation of derived pteridophytic lineages (ferns, lycophytes, sphenopsids, progymnosperms) (Fig. 1b); (3) the appearance of seed plants in the late Devonian and their adaptive radiation in the late Palaeozoic, culminating in a gymnosperm-dominated Mesozoic flora (Fig. 1c); and (4) the appearance and rise of flowering plants in the Cretaceous and Tertiary (Fig. 1d). Two of these four phases of tracheophyte evolution are associated with significant increases in the total species diversity of land plants. Following the establishment of a land flora, the adaptive radiation of the advanced pteridophytes (derived from the earliest tracheophytes), together with the initial expansion of

early seed plants, resulted in a fourfold increase in total species numbers from the late Devonian to the late Carboniferous. The second major increase is associated with the radiation of the angiosperms, resulting in a threefold increase in observed species diversity by the Neogene. The Permo-Triassic boundary marks a period in which known species numbers actually declined by about 20%, and in which the sedimentary record of plant-bearing rocks is poor, at least in Europe and North America. Although the physiognomic and taxonomic changes in terrestrial vegetation accompanying this transition were marked, overall species diversity did not subsequently increase significantly (Fig. 1*b, c*). While a number of time-dependent and time-independent biases influence our estimates of diversity⁴, our summation of the proportional representation of different groups at any one geological time should not be distorted by such biases.

Based on our analysis of tracheophyte history, it is evident that in two cases (the transition of group *a* to *b* and group *c* to *d*) the appearance of a new plant grade was associated with a levelling off and subsequent decline in the number of species in the preceding dominant group. This pattern is similar to that seen for the evolutionary 'succession' of major marine invertebrate groups ('evolutionary faunas') during the Phanerozoic². In parallel, we believe that this pattern suggests the 'competitive displacement' of older, less specialized taxa by newer and presumably more specialized taxa. In a third case (the replacement of group *b* by group *c*), the transition coincides with environmental changes of the late Palaeozoic and earliest Mesozoic. Here, circumstances suggest that the transition is, to some degree, a function of the partial extinction of group *b* followed by the radiation of members of group *c* into the vacated ecological space. It should be noted, however, that species numbers do not necessarily reflect the extent of ecological dominance.

Although stable numbers of species were attained at times in the past, new rounds of diversification accompanied major

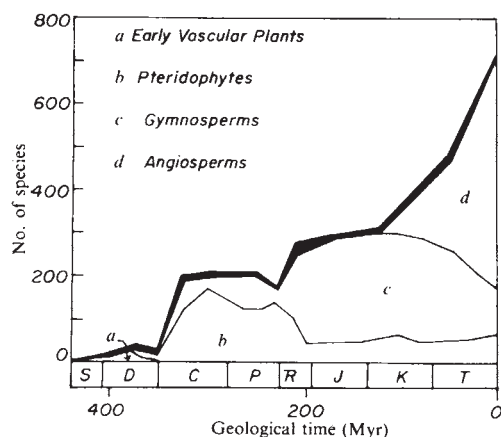


Fig. 1 An analysis of vascular land plant diversity reveals the presence of four groups which have successively dominated the terrestrial flora. Each group is composed of plants sharing a common structural and/or reproductive grade. Following the invasion of land by primitive tracheophytes (group *a*), the remaining three groups successively replace each other, the radiation of one group terminating the dominance of its predecessor. Each radiation involves a major compositional change in the terrestrial flora, and in the cases of groups *b* and *d*, a major rise in overall species number. The transition from group *b* to group *c* is unique in its coincidence with a major period of change in the physical environment. The solid black line at the top of the graph represents non-vascular land plants and *incertae sedis*. Approximately 18,000 fossil plant species citations were compiled and computerized by taxonomic affinity (species, genus, family), age and location. Although the citations were collected worldwide, the majority of the data come from European and North American sources. The data were subjected to a Q-mode factor analysis (ref. 2) using the CABFAC Q-mode vector analysis program⁹⁻¹²: S, Silurian; D, Devonian; C, Carboniferous; Tr, Triassic; J, Jurassic; K, Cretaceous; T, Tertiary.

evolutionary innovations, particularly those involving reproductive features. The data are consistent with ecological theory which suggests that plant species increasingly subdivide their environment through the evolution of more diverse resource use until constrained by the inherent limits of their morphological, physiological and reproductive capabilities⁷. Adaptive radiations of new major groups may involve the removal of one or more of these constraints, permitting new strategies of environmental fragmentation and thus, further diversification. This general pattern is supported by within-flora studies⁴ which similarly suggest that it is innovations in reproductive biology, permitting escape from species saturation in land plant communities, that have characterized the evolution of new plant groups.

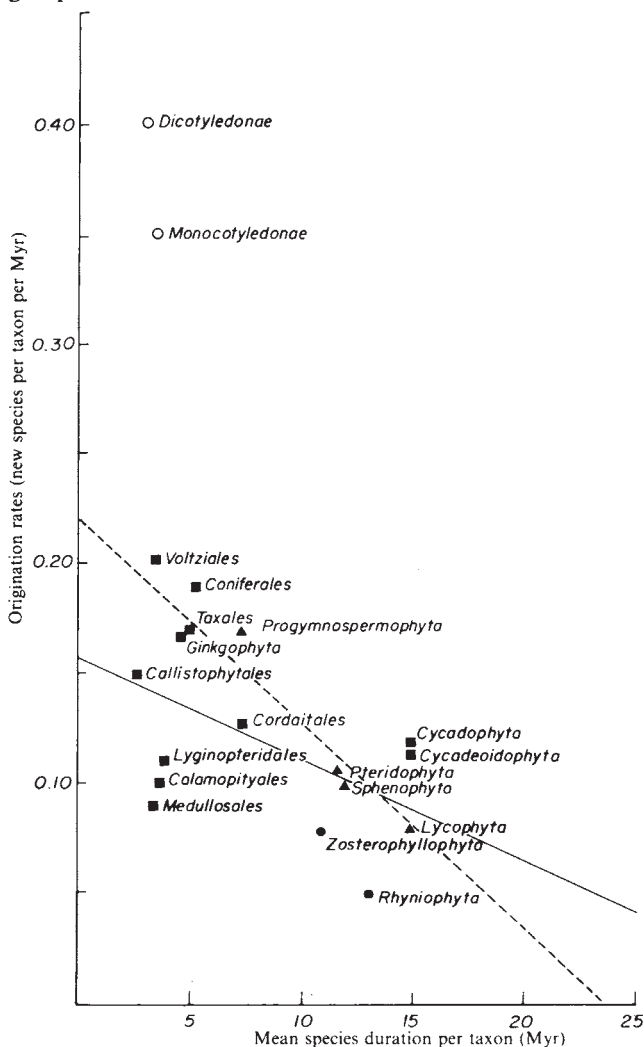


Fig. 2 Summed species origination rates for suprageneric plant taxa versus mean species duration of the same suprageneric taxa. The oldest taxa (rhyniophytes, zosterophyllophytes, lycopods) have the lowest origination rates and longest durations, while the most recent taxa (Monocotyledonae, Dicotyledonae) have the highest origination rates and some of the shortest durations. The dotted line indicates a linear regression of origination rates versus durations for all groups plotted ($r=0.47$); the solid line is a regression of the same data, but excluding the Monocotyledonae and Dicotyledonae ($r=0.49$). Suprageneric groups represented by dots belong to group *a* of Fig. 1, those represented by triangles belong to group *b*, those represented by squares belong to group *c*, and those represented by open circles belong to group *d* of Fig. 1. Origination rates (R) for the various suprageneric groups are based on the following computation: $R = r_s - r_e$, where $r_s = S/Dt$, $r_e = E/Dt$; S is the number of new species, E is the number of extinctions and D is the total diversity (number of taxa in each group) per time interval t . The relationships given are crude estimates of R and D , but are probably an accurate first-order approximation.

In addition to the broad patterns of overall diversity and group replacement, we observe patterns of change in rates of evolution both between and within major groups. Among each successive major group shown in Fig. 1, there is a general trend for an increase in the mean rate of appearance of new species and a decrease in the mean species duration (see Fig. 2). The slowest rates of species origination and longest durations are seen in groups *a* and *b*, while the highest rates and short durations are seen in the angiosperms. Even if the short species durations calculated for the flowering plants falsely result from their relatively recent appearance, their high species origination rates are not influenced by the same bias, and are an order of magnitude above those of the lowest valued group. The rates of species origination and the lengths of species duration also change in a consistent manner within each of the four major groups.

The overall pattern of increasing rates of species origination and decreasing species durations between the major groups are statistically significant. A linear regression of the average species origination rate and the mean species duration for each of the 19 suprageneric groups of Fig. 2 that go to make up the four major groups of Fig. 1 gives $r = 0.47$; elimination of the high values of the angiosperms yields $r = 0.49$ ($n = 17$). Both r -values fall between the 95–99% confidence interval, indicating a high degree of correlation between species origination rates and species durations. Studies indicate that differences in apparent speciation rates have a high correlation with plant breeding strategies and karyotypic variation⁸. Possible plant breeding strategies favouring high speciation rates involve small effective population sizes and specialized mechanisms of pollen and/or propagule dispersal, features commonly found in the most rapidly speciating angiosperm families. By contrast, large effective population size and generalist pollen and propagule dispersal features (commonly observed in gymnosperms) may favour slower speciation rates⁸.

Clearly, the many factors that have influenced the diversification of tracheophytes on the one hand, and marine invertebrates on the other, were both quantitatively and qualitatively different. Similarly, the extent of environmental stability and heterogeneity experienced in the marine and terrestrial habitats are quite distinct. Yet the patterns of diversity observed for these two groups are similar. Both indicate that there have been relatively few changes through time in the structure of the Earth's biota, and that such changes that have occurred have accompanied the radiations of major taxa and have frequently resulted in significant increases in total diversity. Further, following a period of rapid diversification, each evolutionary fauna and flora has tended to approach a levelling-off in the increase in the number of species and ultimately a decline in number as a new group radiates. However, throughout this history, the overall trend has been for a continued increase in total diversity. This suggests that similar types of interactions between developmental potential and ecological constraints may have controlled the evolution of diversity on land and in shallow marine environments.

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Colour-generating interactions across the corpus callosum

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Human vision has the remarkable property that, over a wide range, changes in the wavelength composition of the source light illuminating a scene result in very little change in the colour of any of the objects¹. This colour constancy can be explained by the retinex theory, which predicts the colour of a point on any object from a computed relationship between the radiation from that point and the radiation from all the other points in the field of view (Fig. 1). Thus the computations for colour perception occur across large distances in the visual field. It has not been clear, however, whether these long-range interactions take place in the retina or the cortex. Reports that long-range colour interactions can be reproduced binocularly when one band of wavelengths enters one eye and a different band enters the other² might seem to establish the cortex as the site of the computation. Many observers, however, see very unsatisfactory colour or no colour at all in this binocular situation, suggesting that the cortex may not be the only site at which the computation is carried out, or even the most important site. We have now tested the role of the cortex in a human subject in whom the nerve fibres connecting cortical areas subserving two separate parts of the visual field had been severed, and find that the cortex is necessary for long-range colour computations.

We obtained the subject (J.W.) with the help of Drs Norman Geschwind and Alexander Reeves, and as a preparatory step, we tested his colour vision, which proved to be normal. The subject was a 20-yr-old male who had had intractable epileptic seizures (complex limbic). In 1979 he underwent, in two stages, a complete resection of his corpus callosum, which markedly decreased the number and severity of his seizures. The subject had no obvious intellectual or physical impairment. He had been extensively tested in connection with the callosal cut. In this subject, if the site of the computation is indeed cortical, events in one visual half-field should have no influence on the appearance of objects in the other half-field. If an influence is found, the computation responsible for that influence must be retinal.

Our objective was to construct a visual scene in which there was one region, of constant luminous flux and wavelength composition, whose colour was to be reported on. This test region would span the midline of the visual field; a large second region whose illumination could be varied would be confined to one visual half-field or to the other.

We finally adopted the set-up shown in Fig. 2, where F, the point of fixation, lay within a test spot, T (Color-Aid paper RV Tint 1). A Mondrian³ of variously coloured Munsell papers lay to the left of the test spot and hence was entirely in the subject's left visual field (Fig. 2a). The parts of the field to the right of the borders of the Mondrian and test spot were black velvet. To keep the Mondrian in the subject's left visual field, we set the fixation point at 3.7° to the right of the right-hand border of the Mondrian. Beyond the experimental display, in the subject's extreme visual periphery all the surfaces in the room were low-reflection blacks.

Fig. 1 The present method of computation⁵ differs from those of previously published versions of the retinex theory⁶. To predict the perceived colour of an area in any visual scene, the computation shown here is performed. The visual field is broken into unit areas. The relative reflectance, R , of the target area, i , is computed with respect to some other area, j , along a path drawn between the two areas using the formula shown, where Λ designates the particular waveband (long, middle or short) and I is the intensity. The threshold operation on the ratios along the path is included in order to remove the effects of non-uniform illumination over the scene: variations gradual enough to be below threshold are dropped out. All others are considered significant and contribute to the computation. The average of many such computed relative reflectances is taken in order to determine the value we define as average relative reflectance at area i . Conceivably, this average of relative reflectances (not fluxes) could be taken over every area in the visual field, but as few as 100–200 is usually sufficiently accurate. The average is taken over areas from the entire visual field and not just those nearby; experiments indicate there may be nearly as much contribution from distant areas as nearby ones. As the above computation is carried out three times, once for each of the three wavebands, three numbers (designators) become associated with each unit area. These designate a point in a three-dimensional colour space, a point which proves to be invariant with large changes in quantity and composition of illumination of the field of view. Experimentally it is found that if two points in any visual scene are the same colour (even when, because of non-uniform illumination, the wavelength composition reflected from them may be very different), they will be represented at the same point or closely adjacent points in this colour space.

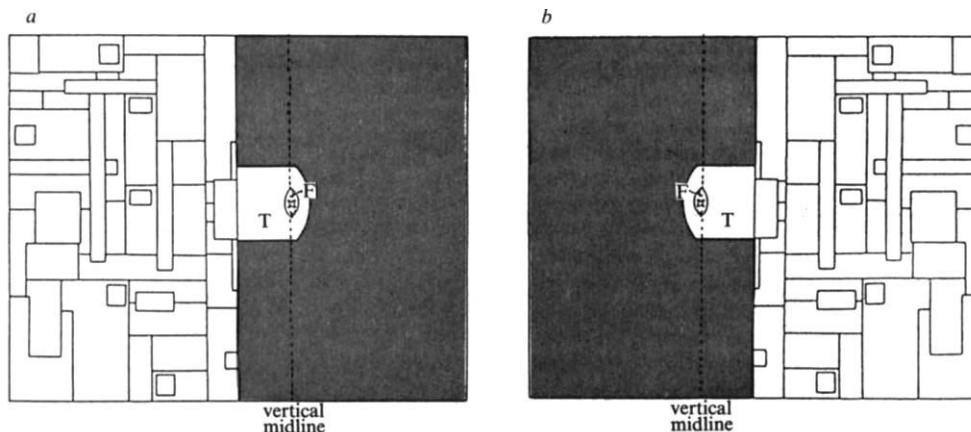
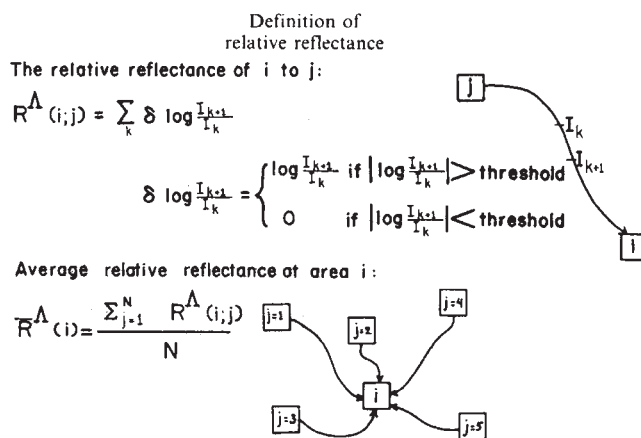


Fig. 2 A Mondrian collage of variously sized coloured matte papers, with a test spot, T, which is bordered on three sides by black velvet. A $0.75^\circ \times 1.7^\circ$ window in the test spot is at the fixation point, F. Viewed directly (a), the right-hand border of the Mondrian lies at 3.7° to the left of the vertical midline; viewed in a mirror (b), the Mondrian lies 3.7° to the right of the vertical midline. Behind the window at fixation point, F, lies a rotating disk which introduces bilaterally symmetrical letters into the window at the rate of 3.5 s^{-1} .

The fixation region was an aperture, $0.75^\circ \times 1.7^\circ$, behind which lay a rotating black disk from which a ring of bilaterally symmetrical letters (such as A, O, M, H) had been cut out. The background for the cut-out letters was a piece of paper identical to that used for the test spot. As the disk rotated, any given letter was replaced in the aperture by the one above it at the rate of 3.5 s^{-1} . We used a Mondrian instead of a uniform field, and a moving fixation target instead of a fixation point, in order to reduce the risk of after-images⁴. Another reason for using the Mondrian is that in different lighting conditions the colours (but not the brightnesses) remain virtually unchanged compared with the violent changes in colour that a uniform field would exhibit. The subject was asked to read each of the letters aloud as it appeared, and on hearing the word "colour" barked at him by the experimenter (E.H.L.), to report the colour in the test region.

The whole display (test spot, Mondrian, velvet) was illuminated by three tungsten slide projectors each containing a colour filter, the curves of which are shown in Fig. 3, and a choice of neutral density filters in which holes had been cut; these holes, when projected in focus from the slide position, matched exactly the shape and size of the test spot. Thus when the neutral filters were changed, the illumination of the Mondrian changed, but the illumination of the test spot remained constant. Projector intensities and neutral filters were so chosen by the

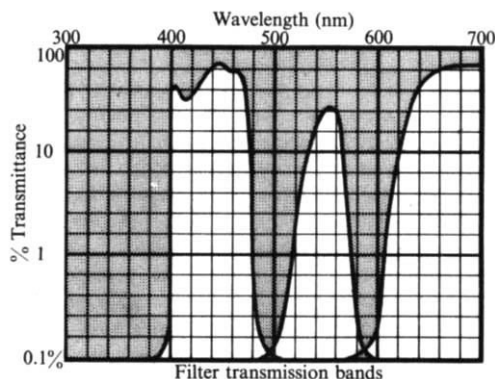


Fig. 3 The transmission bands of filters used to illuminate the display in Fig. 2. The measured test spot radiance, both when it was white and when it was purple, was $0.21 \text{ W sr}^{-1} \text{ m}^{-2}$ in the long waveband, $0.19 \text{ W sr}^{-1} \text{ m}^{-2}$ in the middle waveband and $0.073 \text{ W sr}^{-1} \text{ m}^{-2}$ in the short waveband. A piece of white paper (Color-Aid 91% reflectance) in the Mondrian in the condition in which the test spot was purple measured 0.30, 0.83 and $0.21 \text{ W sr}^{-1} \text{ m}^{-2}$ in the long, middle and short wavebands. In the situation in which the test spot was white, neutral density filters of 1.0, 1.7 and 1.2 were inserted in the respective waveband illuminators for the Mondrian only. (The test spot illumination remained constant throughout.)

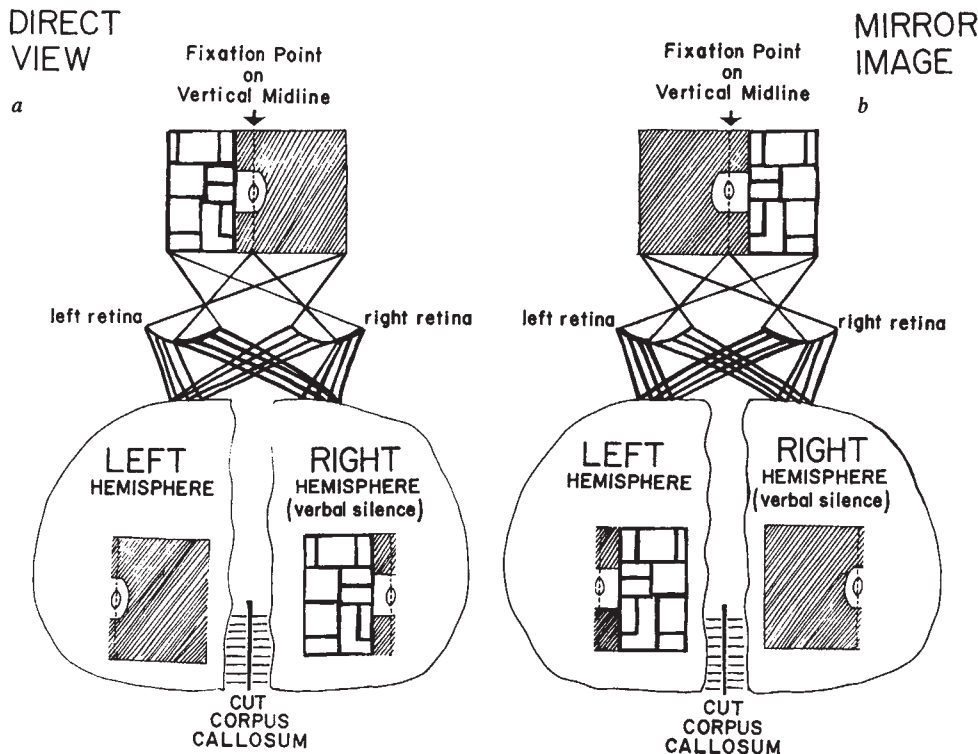


Fig. 4 A schematic representation of the information reaching the left and right hemispheres of the subject with the cut corpus callosum when he views the display depicted in Fig. 2.

computations of retinex theory (Fig. 1) that with neutral filters in place the test spot was a uniform chalky white, whereas with no neutral filters it was a uniform deep purple—although the flux from the test spot had in fact not changed at all. Most naive subjects consistently reported the test spot to be white in the first situation and purple in the second situation. A few called one “light purple” and the other “dark purple”, but any one subject’s reports were consistent.

The subject is right-handed and it had been previously determined that his speech centre was in his left hemisphere (A. Reeves, personal communication). He could therefore verbally describe things seen in his right visual field, but not things seen in his left visual field.

When the test apparatus was arranged so that the Mondrian was entirely in the left visual field with the test spot spanning the vertical midline (Fig. 4a), the split-brain subject consistently reported that the test spot was “white” regardless of the illumination of the Mondrian, even though to a normal observer it appeared purple when the Mondrian was fully lit and white when the lighting of the Mondrian was appropriately attenuated by the set of neutral filters. When he viewed the set-up in a mirror (Fig. 4b), the Mondrian was in his right visual field and the test spot again spanned the vertical midline. Now, with the Mondrian lit, he first stated that he saw “all colours, green and brown”, but on questioning it turned out that he was describing the entire Mondrian, something he had apparently not been able to do when it was presented to his nonverbal hemisphere. Thereafter, having been instructed to describe only the test spot, he reported it as “purple” when the Mondrian was lit without attenuators and “white” when the lighting of the Mondrian was attenuated by neutral filters, in complete agreement with the appearances to a normal observer.

We alternated viewing sessions so that the Mondrian was entirely in the right visual field (Fig. 4b) or entirely in the left visual field (Fig. 4a). When the Mondrian was in his left visual field, the subject almost always called the test spot “white”, occasionally “purple”, but not correlated at all with the appearance to a normal observer. When he viewed the set-up in a mirror, thus transposing the Mondrian to the right visual field,

his responses were always in accord with the appearance to a normal observer.

It could be objected that the part of the test spot reported on by the subject in the first (mirrorless) situation was farther from the Mondrian, by about 4°, than in the control situation, in which the two abutted each other; and that as a consequence the influence of Mondrian on test object might have fallen to insignificance in that 4° distance. For two reasons we are convinced that this was not so. First, when the Mondrian was confined to the left visual field, and then a 3° circular test spot, entirely surrounded by black velvet, was placed at varying distances along the horizon in the right visual field, normal observers continued to see a clear alternation from white to purple in the two lighting situations, even when the separation was extended to 30° or when, in simulation of what the subject presumably saw in configuration *a* of Fig. 4, the test spot was a 1° × 3° crescent 4° from the right-hand border of the Mondrian. Second, to a normal observer the test spot, in either of the mirror symmetry situations, appeared absolutely uniformly purple or white (as predicted by retinex theory).

Since J.W. gave normal responses when the Mondrian was in his right visual field, his visual system is clearly capable of generating the long-range interactions that occur in a normal observer. What he failed to do was to transfer the information which would enable him to make a computation based on the whole field of view across the vertical midline. Therefore the calculation could not have occurred solely or largely in the retina and must have occurred in the cortex, where regions representing the two halves of the visual field are joined by the corpus callosum.

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RNA synthesis dependence of action potential development in spinal cord neurones

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The role of transcription in the development of electrical properties of neuronal membranes has been largely unexplored. To study the molecular events which result in the expression of these properties it is useful to describe the timing of the underlying RNA synthetic events. For example, the timing of the transcription involved in denervation-induced action potentials in frog slow muscle fibres¹ and brain extract-induced sodium channels in chick muscle cells² has been investigated. Previous studies of *Xenopus laevis* spinal neurones have established that the timing of the development of the neuronal action potential ionic dependence in dissociated cell cultures parallels that seen *in vivo*³⁻⁶. This culture system, therefore, allows the determination of transcription-dependent periods necessary for the development of membrane properties known to have *in vivo* relevance. In the study described here, actinomycin D was used to examine the timing of the RNA synthetic events necessary for (1) neurite outgrowth and (2) development of the ionic dependence of the action potential. I report that inhibition of transcription at an early stage specifically blocks the appearance of the mature sodium-dependent action potential without affecting either neurite outgrowth or the development of delayed rectification.

Many cell types differentiate in dissociated cell cultures prepared from neural plate-stage *Xenopus* embryos; neurones are recognized only after the extension of neurites, which begins ~6 h after plating. When actinomycin D, which inhibits DNA-directed RNA synthesis⁷, at 2.5 $\mu\text{g ml}^{-1}$ was added to the cultures earlier than 1.5 h after plating (19 h after fertilization), there was no obvious morphological differentiation of any cell type. However, chronic exposure to actinomycin D begun between 1.5 and 4 h after plating inhibited neuronal incorporation of ³H-uridine (Fig. 1) but allowed extension of neurites from cells at ~6 h, as observed for untreated cells. Over the next 2.5 days the range of morphological variability seen in neurites from actinomycin D-treated neurones was similar to that in control cells, although there were fewer neurones in the inhibited cultures (a reduction in the range of 20 to 80%). There were also fewer differentiated cells of other types in the actinomycin D-treated cultures.

To determine whether the effect of actinomycin D on neurite outgrowth was stage-dependent, the inhibitor was added at various times after plating to cultures made from embryos at stages between the late gastrula and late neurula. Actinomycin D could be added at progressively earlier times after plating, as cultures were prepared from later stage embryos, and still allow neurite extension. For example, late neurula embryos corresponding to 19 h after fertilization were cultured directly into actinomycin D and still extended neurites at times comparable to controls. Thus the RNAs necessary for neuronal survival or neurite elaboration seem to be transcribed before 1.5 h after culturing, that is, before 19 h after fertilization. In PC12 cells, initiation of *de novo* neurite outgrowth also requires transcription before RNA synthesis-independent growth can proceed⁸. The experiments reported here do not exclude the possibility that neurones do not survive actinomycin D treatment begun prior to 19 h after fertilization. However, use of reversible metabolic inhibitors for protein synthesis in *Xenopus* neurones⁹ and for RNA synthesis in PC12 cells¹⁰ suggests that it is possible for cultured cells to survive periods of synthesis inhibition, and initiate or resume neurite outgrowth on resumption of synthesis.

The duration of the action potential is an indication of its ionic dependence, and thus the developmental maturity of these spinal cord neurones⁴. Young cells from control cultures have action potentials of long and variable duration which are primarily calcium-dependent. At slightly later times the action potential duration decreases and an overshooting sodium component and a small calcium component are present. Late-stage action potentials are very brief and primarily sodium-dependent. Figure 2 illustrates the action potential duration as a function of age in culture. The durations seen in control neurones declined whereas the action potentials of the actinomycin D-treated neurones examined over the same time period did not decrease in duration or variability. These data suggest that actinomycin D-treated neurones maintain a primarily calcium-dependent action potential even at late times in culture.

The ionic dependence of the action potentials was examined directly by ionic substitutions and addition of blocking agents to the perfusion saline. At early times in culture both control and actinomycin D-treated neurones exhibited overshooting action potentials that were primarily calcium-dependent events with a slow sodium component (Fig. 3A, B). Control neurones examined at 50 h in culture showed short-duration sodium-dependent action potentials (Fig. 3C). In contrast, the long-duration action potentials of the inhibited neurones were primarily calcium-dependent, even at 50 h in culture. The sodium component at this time appeared unchanged from that seen in control and experimental neurones at early times (Fig. 3D). Thus, the development of the ionic dependence of the

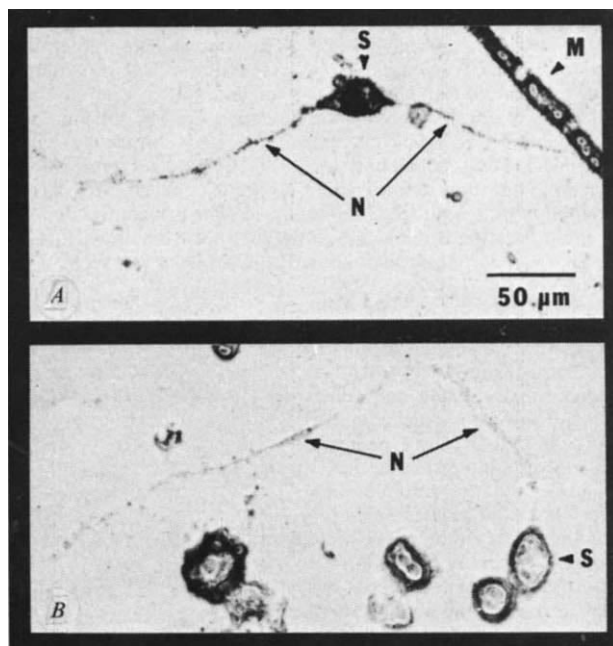


Fig. 1 Neuronal morphology and incorporation of ³H-uridine in control and actinomycin D-treated cultures. **A**, Control neurone viewed by brightfield illumination at 24 h *in vitro* shows dense labelling of the soma (S), and neurites (N). **B**, A neurone from an actinomycin D-treated culture is morphologically similar to the control at 24 h, but shows no radioactive labelling above background. As the number of neurones does not increase after 24 h and actinomycin D is chronically present, it is likely that neurones examined physiologically at times after 24 h exhibit comparable levels of inhibition. Dissociated cell cultures were prepared from neural plate-stage embryos^{4,14}, in a simple, fully defined medium⁹. To determine the level of inhibition by actinomycin D, control cultures were exposed to 5 $\mu\text{Ci ml}^{-1}$ ³H-uridine from 2 to 24 h, at which time they were fixed and processed for autoradiography. Experimental cultures were prepared similarly, with the addition of 2.5 $\mu\text{g ml}^{-1}$ actinomycin D 15 min before exposure to ³H-uridine. Actinomycin D at 2.5 $\mu\text{g ml}^{-1}$ also inhibited >95% of the incorporation of ³H-uridine into the TCA-precipitable cell fraction from these cultures.

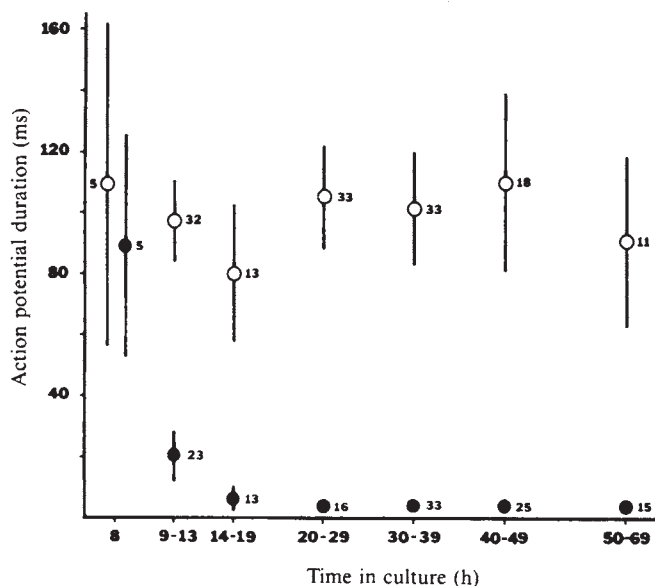


Fig. 2 The action potential duration as a function of time in culture. Action potentials were elicited in response to short depolarizing current pulses in neurones between 8 and 69 h after plating. Each point was determined by averaging action potential durations in normal saline (NaCl, 40 mM; CaCl₂, 10 mM; HEPES, 5 mM (pH 7.4); KCl, 3 mM) for the number of neurones indicated next to each point on the graph; bars indicate s.e.m. The resting potential of both control and actinomycin D-treated neurones ranged between -50 and -90 mV. The control action potential duration (filled circles) at 8 h is long and exhibits a large variability. Both the duration and variability decrease with increasing age, indicating a developmental switch from primarily calcium to sodium ionic dependence. Neurones treated chronically with actinomycin D first added between 19 and 21.5 h after fertilization have long-duration action potentials (open circles) with large variability throughout the time examined. The 8-h control action potential duration is not significantly different from any of those seen in neurones from inhibited cultures from 8 to 69 h.

action potential of the inhibited neurones seems to have been arrested at an early developmental stage. Selective survival cannot account for these results as none of the untreated neurones at late times showed long-duration calcium-dependent action potentials. These results suggest that the RNAs necessary for acquisition and maintenance of a calcium action potential with an early sodium component are synthesized earlier than 19 h after fertilization but that the maturation of the fast sodium component requires a period of RNA synthesis that extends beyond 19 h.

The persistence of long-duration action potentials of mixed ionic dependence seen in inhibited neurones could be explained by the effect of RNA synthesis inhibition on any of several developing properties of the neurones. Inhibition may prevent both an increase in sodium conductance during development and simultaneously a decrease in calcium conductance. Another possibility is that actinomycin D affects the development of outward currents involved in repolarization of the action potential. To investigate this last possibility, delayed rectification was examined. A voltage-dependent conductance increase yielding delayed rectification was present at 7-10 h and increased by ~50% between 10 and 13 h in both control and experimental cultures (Table 1). These results indicate that the development of delayed rectification is not grossly affected by the presence of actinomycin D, and that the increase in voltage-dependent delayed rectification does not have a major role in determining the duration of the action potential in actinomycin D-treated neurones. The input resistance seems neither to change over the time period examined nor to be altered by the presence of actinomycin D.

Does the RNA synthesis inhibition of the impulse show a stage dependence similar to that seen for neurite outgrowth? To address this question, inhibition of RNA synthesis was initiated at progressively later times between 21.5 and 35 h after fertilization. This allowed development of neurones having progressively more mature action potentials, an increasing percentage of the inward current carried by sodium and a decreasing calcium component. Thus, the development of the impulse requires a period of RNA synthesis that extends for 30-35 h after fertilization and it seems to be affected in a stage-depend-

Fig. 3 Ionic dependence of action potentials from control and actinomycin D-treated neurones at early and late times in development; records from four cells. Upper trace in all records indicates the current amplitude and the zero potential; lower trace indicates membrane potential. Time calibration is noted below each trace. **A** and **B** illustrate action potentials of neurones at early times in control and actinomycin D (ACD)-treated cultures (13 h) which have a long-duration, overshooting calcium component that is blocked by cobalt. A slow sodium component can be detected in the presence of cobalt. **C** Shows a mature sodium-dependent action potential from a control neurone at 50 h. The short-duration action potential is present in normal saline and in saline plus cobalt but cannot be elicited when external sodium is removed. In contrast, the action potential of the actinomycin D-treated neurone at 50 h (**D**) is still primarily calcium-dependent, similar to the action potential from both control and actinomycin D-treated neurones at early times (**A** and **B**), with a slow sodium component that is present in cobalt but is blocked on removal of sodium. Accurate determination of the voltage necessary for impulse initiation was not possible from these records in which current was passed and voltage recorded with the same electrode.

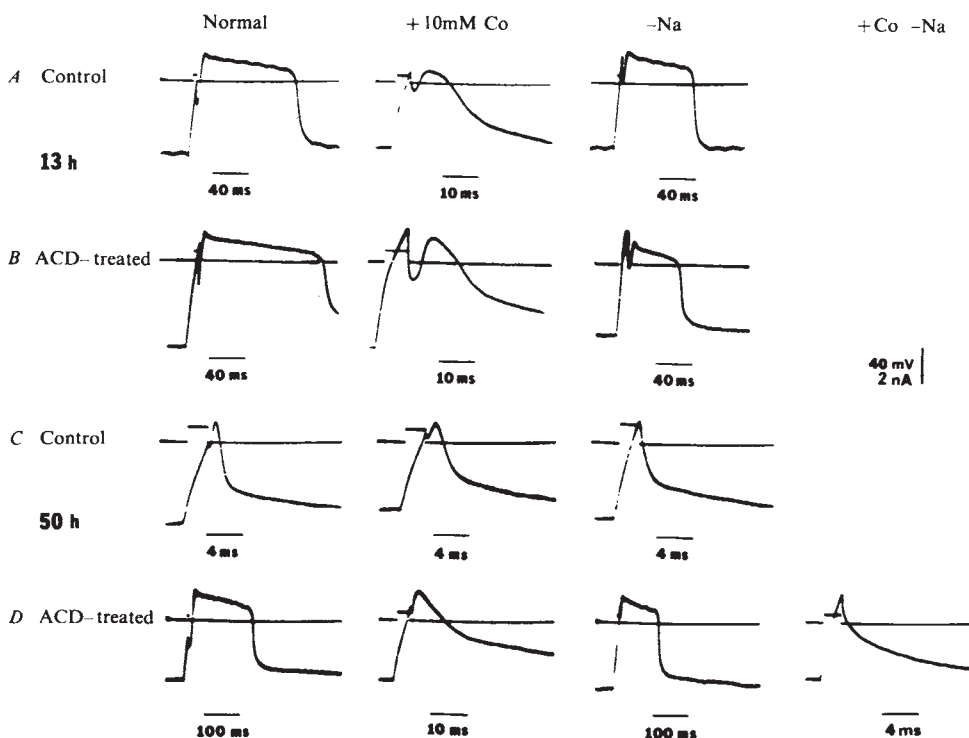


Table 1 Rectifying conductance develops similarly in neurones from control and experimental cultures

Hours in culture	Rectifying conductance (%)	
	Control	Actinomycin D-treated
7–10	0.39 ± 0.02 (7)	0.33 ± 0.06 (8)
13–15	0.53 ± 0.10 (5)	0.58 ± 0.04 (6)
19–21	0.49 ± 0.05 (8)	0.50 ± 0.06 (10)
29–31	0.50 ± 0.05 (4)	0.53 ± 0.08 (5)

Values are mean ± s.e.m. (*n*). Current-voltage curves were constructed by measuring the voltage response of the membrane to long current pulses (800 ms) of small amplitude (–10 to +20 pA) with a single electrode that was in balance before and after the measurements were made. The outward currents activated within 30 ms and did not inactivate within 800 ms. An increase in conductance due to activation of the late outward current channels was seen in both control and actinomycin D-treated neurones between 10 and 13 h, indicating the normal development of delayed rectification in the presence of actinomycin D. The per cent conductance due to delayed rectification (rectifying conductance, %) is defined as $G_R - G_P / G_R$, where G_P is the slope of the *I*-*V* plot in the hyperpolarizing quadrant and G_R (conductance after rectification) is the slope of the best fit line in the depolarizing quadrant of the points that deviate from G_P . Inward currents were suppressed pharmacologically with 10^{-6} g ml⁻¹ tetrodotoxin and 10 mM CoCl₂. The conductances measured in actinomycin D-treated and control neurones at 7–10 h are not significantly different (Student's *t*-test). Experimental and control conductances between 13 and 31 h also fail the significance test. However, experimental and control conductances at 7–10 h are significantly different from the pooled conductances at later times.

dent manner by actinomycin D. Although the possibility has not been excluded, it seems unlikely for two reasons that actinomycin D affects nontranscriptional events, for example, inhibition of DNA and protein synthesis: (1) Blair has shown⁹ that direct inhibition of protein synthesis affects the same properties reported here with a stage dependency 3 h later in development; (2) the cultures were treated with a relatively low concentration of actinomycin D¹¹.

Two of the properties examined—neurite outgrowth and the development of delayed rectification—seem to mature normally in these cells during inhibition of RNA synthesis begun 2–4.5 h before neurite outgrowth takes place. This finding is consistent with the observations that morphological differentiation of *Drosophila* and murine superior cervical ganglion neurones requires transcription before the first axons are seen, after which time the neurones enter an actinomycin D-insensitive period during which axon elongation proceeds^{12,13}. Two other properties, the resting potential and input resistance, also appear unaltered in the *Xenopus* neurones exposed to actinomycin D. In contrast, maturation of the fast sodium action potential apparently requires an extended period of RNA synthesis. The finding that the calcium action potential is similar at both 8 and 69 h during exposure to actinomycin D raises the possibility that the metabolic turnover of RNAs and proteins responsible for maintenance of this phenotype is rather slow. Examination of RNA synthesis-dependent periods in simple developing systems such as this one may provide an understanding of the molecular mechanisms by which the early programme of differentiation is elaborated.

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Transmitter-like action of ATP on patched membranes of cultured myoblasts and myotubes

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The concept of purinergic neurotransmission, first proposed by Burnstock¹, has been confirmed in various cell types. We show here, by the patch-clamp method², that external ATP in micromolar concentrations (1–100 μM) activates cation channels in the membranes of fusion-competent myoblasts and myotubes. In cell-attached membrane patches of myoblasts and myotubes the mean number of simultaneously activated channels increases with time after external ATP application. In myoblasts only one population of channels having a mean single-channel conductance of $\gamma = 43$ pS was found, while in myotubes two populations with $\gamma_1 = 48$ pS and $\gamma_2 = 20$ pS were observed. Treatment of myotube membranes with acetylcholine (ACh) or carbachol resulted in two populations of channels which had conductance values and voltage-dependent mean channel lifetimes similar to those produced in response to ATP. The results show that embryonic skeletal muscle cells contain cation channels sensitive to ATP and provide evidence for a neurotransmitter-like action of ATP on these cells.

Myoblasts were prepared from 12-day-old chicken embryos and cultured as previously described³. The cells were maintained in a medium Ca²⁺ concentration of 10^{-7} M for 48 h, during which time they achieved fusion competence; raising the Ca²⁺ concentration at this time to 1.4 mM results in rapid, synchronous fusion. Thirty minutes before measurements the essentially Ca²⁺-free culture medium was replaced with the following solution (in mM): NaCl 137, KCl 5.4, CaCl₂ 1.4, MgCl₂ 0.8, HEPES 10, pH 7.4, glucose 10. The Ca²⁺ concentration was increased at this point to allow measurements in more physiological conditions and to improve the pipette-cell attachment; Ca²⁺-free solutions gave similar results. Pipettes used for recording single-channel currents were filled with solutions containing ATP, ACh or carbachol. Experiments were performed at 20–22 °C.

In myoblasts, addition of 1–100 μM ATP to the pipette solution resulted in the induction of single elementary current events of similar amplitudes (Fig. 1). At ATP concentrations >10 μM, the mean number of channels activated simultaneously increased visibly with time (Fig. 1A, B). From the linear current-voltage relationship in the voltage range between the resting potential (V_m) and $V_m - 120$ mV, a zero-current potential of $V_m + 38 \pm 6$ mV (mean ± s.e.; *n* = 6) was derived by extrapolation. The single-channel conductance, γ , was 43 ± 3 pS (*n* = 6). The channel showed no detectable selectivity between Na⁺ and K⁺, it did not seem to be significantly permeable to Ca²⁺ and was not blocked by raising the extracellular Ca²⁺ concentration to 16.4 mM (Fig. 2A). Figure 2 also shows that the mean current in the presence of ATP increased with hyperpolarizing membrane potential as a result of several voltage-dependent processes. First, there was an increase in single-channel current, due to its ohmic behaviour (straight lower line in current record). Second, there was a potential-dependent

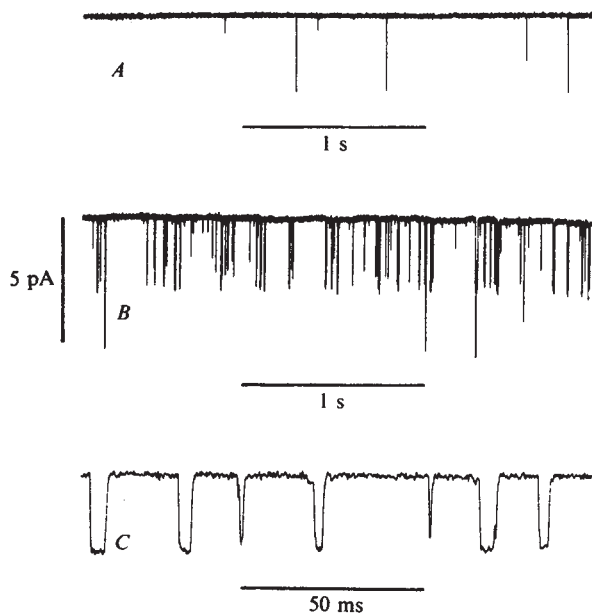


Fig. 1 ATP-activated currents in embryonic chicken myoblasts recorded from a cell-attached patch. *A*, *B*, Consecutive traces from the same patch at a constantly applied pipette potential of $V_m - 20$ mV, where V_m is the resting potential (not measured directly) and the applied voltage refers to the additional potential difference (inside minus outside) of the membrane patch. Records in *A* and *B* were obtained at 40 s and 20 min, respectively, after a seal resistance of 23 G Ω had been achieved. *B* Shows in three cases the simultaneous activation of two elementary channels. The recording pipette was filled with the bathing solution (see text) containing in addition 100 μ M ATP. *C* shows a time-expanded part of *B*. One class of elementary events is evident with a mean lifetime of $\tau = 3.3$ ms. From the slope of the current (I)-voltage relation measured in the potential range between V_m and $V_m - 120$ mV, we found a single-channel conductance of $\gamma = 43$ pS. By extrapolating the I - V relation, a zero-current potential of 42 mV positive to the resting potential was obtained; the resting potential, measured independently, was ~ -50 mV.

increase in the frequency of channel openings (compare also insets *a* and *b* of Fig. 2*B*). Third, the mean lifetime of the channel open state, τ , became larger (see shift of corner frequencies, $f_c = 1/2\pi\tau$, in Fig. 2*B*). τ increased exponentially with increasing membrane potential in the hyperpolarizing direction. Thus, τ increased about e -fold for a 70-mV change in the membrane potential. A similar voltage dependence of τ is reported for ACh-activated cation channels at the neuromuscular endplate^{4,5}. Activation of this channel was not observed in response to ADP, AMP or adenosine, at concentrations up to 100 μ M.

Experiments were also performed on 96-h cultured myotubes. These cells are the precursors of skeletal muscle fibres and possess muscle-specific proteins. In these cells, two current states of ATP-activated channels were observed (Fig. 3*A*). We suggest that the elementary current states, i_1 and i_2 (Fig. 3*A*), arise from two different channel populations and not from two different states of the same channel, as kinetic transitions between i_1 and i_2 were not observed. The mean lifetime, τ_1 , of the channel population of larger amplitude i_1 was always smaller than τ_2 for the population of smaller amplitude i_2 (Fig. 3*A*). Similar channel activation was observed in response to carbachol (Fig. 3*B*) and ACh (result not shown), but in this case τ_1 was larger than τ_2 . The single-channel conductances obtained in the presence of carbachol were $\gamma_1 = 30$ pS and $\gamma_2 = 48 \pm 4$ pS, with corresponding mean open lifetimes of $\tau_1 = 3.5 \pm 0.5$ ms and $\tau_2 = 1.3 \pm 0.3$ ms ($n = 4$) at the resting membrane potential. The mean ratio i_1/i_2 of 1.8 for the carbachol-induced single-channel currents was lower than that for the ATP-activated elementary currents ($i_1/i_2 = 2.4$). The phenomenological appearance of these two channel populations is similar to that described in non-innervated rat muscle cells in response to ACh⁶. However, in contrast to embryonic rat muscle, we have not observed subconductance states in addition to the 'main' state of the channel conductances γ_1 and γ_2 .

Our results suggest the presence of an ATP receptor in cultured embryonic muscle cells. To our knowledge this is the first such report; however, potentiation of ACh currents by ATP has been observed in rat diaphragm muscle⁷. ATP has also been shown to produce membrane potential changes in frog heart muscle, although in this system, adenosine, AMP, ADP and ATP were equipotent⁸. Two possible functional roles for ATP present themselves. First, ATP receptors, distinct from ACh receptors, may exist on muscle cell membranes and second, the ATP receptors could be embryonic precursors of ACh receptors. We cannot at this stage speculate further but it is noteworthy that the ACh and ATP molecules have very similar charge characteristics⁹.

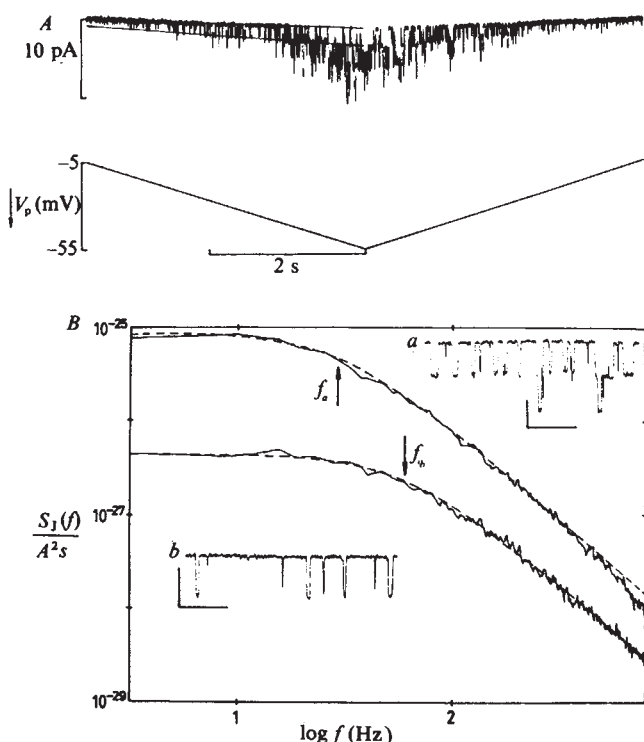


Fig. 2 Voltage dependence of ATP-induced single-channel currents in a cell-attached patch of a myoblast cell. *A*, The applied pipette potential (V_p) was changed in a ramp-like fashion (lower part) between -5 and -55 mV negative to the resting potential with a periodicity of 0.14 Hz. From the I - V relationship of the base line and the single channel (drawn lines in the current trace) one obtains a seal resistance of 25 G Ω , a single-channel conductance of 26 pS and, by extrapolation, a zero-current potential of 23 mV positive to the resting potential. The bathing solution was the same as that described for Fig. 1, but the pipette solution had the following composition (mM): ATP 0.01, NaCl 75, CaCl₂ 16.4, MgCl₂ 0.8, HEPES 10, pH 7.4, glucose 10. *B*, Power spectral densities $S_1(f)$ of ATP-induced current fluctuations at two membrane potentials. Parts of the corresponding current traces from which $S_1(f)$ was calculated are shown as insets. Inset *a* shows the current record at $V_m - 50$ mV and *b* that at $V_m - 20$ mV. Calibration bars for current traces are 3 pA and 50 ms. The spectra were calculated on-line with a Hewlett-Packard 5420A digital signal analyser; the lower roll-off frequency was 0.1 Hz. The broken lines are fits to the theoretical spectrum calculated for a first-order kinetic behaviour of the channel due to $S_1(f) = A/[1 + (f/f_c)^2]$. The corner frequencies ($f_c = 1/2\pi\tau$, marked by arrows) derived from the spectra were: for potential $V_m - 50$ mV, $f_c = f_a = 58$ Hz and for $V_m - 20$ mV, $f_c = f_b = 32$ Hz. From the corner frequencies we derived the following mean lifetimes of the channel open state: $\tau_a = 2.7$ ms and $\tau_b = 5.0$ ms.

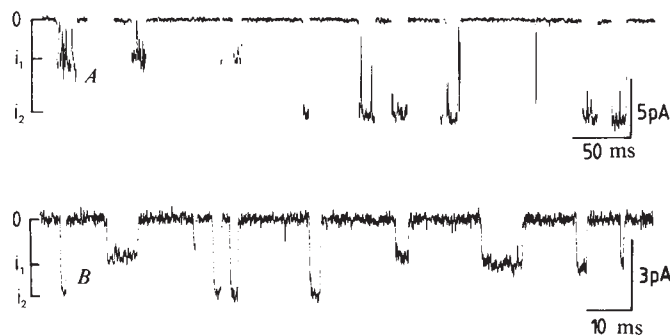


Fig. 3 Comparison of ATP(A)- and carbachol(B)-induced current fluctuations on chicken myotubes in cell-attached conditions of the micropipette. Two distinct classes of elementary currents (i_1 , i_2) are observed in both cases. The electrolyte compositions of the bathing medium and pipette solution were the same as those in Fig. 1 legend. A, 5 μ M ATP was added to the pipette solution. The record was taken 15 min after obtaining a G Ω seal. The membrane potential applied to the patch was -100 mV. From the corresponding I - V curves for i_1 and i_2 we found $\gamma_1 = 20$ pS and $\gamma_2 = 48$ pS and a common zero-current potential of 42 mV positive to the resting potential. The mean lifetimes of the two classes of elementary events were: $\tau_1 = 1.3$ ms and $\tau_2 = 2.2$ ms, both of which increased about e -fold for a 70-mV change in the membrane potential in the hyperpolarizing direction. B, 5 μ M carbachol was added to the pipette solution, and the measurement taken at the resting potential. The single-channel conductances were $\gamma_1 = 25$ pS and $\gamma_2 = 44$ pS and the zero-current potential was 45 mV positive to the resting potential. The corresponding mean lifetimes of the open states were $\tau_1 = 3.2$ ms and $\tau_2 = 1.1$ ms. The mean lifetimes increased e -fold for a 120 mV change of the membrane potential in the hyperpolarizing direction.

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GABA affects the release of gastrin and somatostatin from rat antral mucosa

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γ -Aminobutyric acid (GABA) is regarded as the major inhibitory neurotransmitter in the central nervous system of vertebrates¹⁻³. GABA exerts its inhibitory actions by interacting with specific receptors on pre- and postsynaptic membranes⁴⁻⁶ and has been shown to inhibit somatostatin release from hypothalamic neurones *in vitro*⁷. Concepts of innervation of the gastrointestinal tract⁸ have been expanded by recent studies which suggest that GABAergic neurones are not confined solely to the central nervous system but may also exist in the vertebrate peripheral autonomic nervous system⁹. Jessen and coworkers^{9,10} have demonstrated the presence, synthesis and uptake

of GABA by the myenteric plexus of the guinea pig taenia coli, and have documented the presence of glutamic acid decarboxylase (GAD) in isolated myenteric plexus. This enzyme is responsible for the conversion of glutamic acid to GABA in GABAergic neurones. The possibility that GABA may have a role in neurotransmission or neuromodulation in the enteric nervous system of the vertebrate gut has been suggested by several investigators¹¹⁻¹³. Furthermore, GABA receptors have been demonstrated on elements of the enteric nervous system^{14,15}. The effects of GABA on gastrointestinal endocrine cell function have not been examined. We report here the effects of GABA on gastrin and somatostatin release from isolated rat antral mucosa in short-term *in vitro* incubations.

Administration of the lowest dose of GABA (10^{-9} M) to incubated antral mucosal fragments resulted in a 146% increase in gastrin release above control values: 25.3 ± 2.9 and 62.3 ± 11.0 ng gastrin per mg protein for control and 10^{-9} M GABA, respectively ($P < 0.01$; Fig. 1a). 10^{-8} M GABA caused maximal stimulation of gastrin release (103.1 ± 13.2 ng per mg protein) and resulted in a 307.4% increase above control media gastrin content ($P < 0.001$). GABA-stimulated gastrin release with 10^{-7} and 10^{-6} M GABA was maintained at levels significantly above control values (87.4 ± 8.4 and 87.1 ± 8.0 ng gastrin per mg protein, respectively; $P < 0.001$).

In contrast to the stimulatory effect of GABA on gastrin release, antral somatostatin was inhibited in a dose-dependent manner with increasing concentrations of GABA in the incubation medium (Fig. 1b). Maximal inhibition of somatostatin release was observed with 10^{-6} M GABA. At this concentration, GABA inhibited basal somatostatin release by greater than 90%: 0.70 ± 0.16 and 0.06 ± 0.01 ng somatostatin per mg protein for control and 10^{-6} M GABA, respectively; $P < 0.01$. Half-maximal inhibition of somatostatin release by GABA was achieved with 10^{-8} M GABA.

The effects of GABA on *in vitro* antral gastrin and somatostatin release were explored further by using the specific GABA-receptor antagonist, bicuculline¹⁷. Figure 2 demonstrates the effects of increasing medium concentrations of bicuculline (10^{-8} to 10^{-6} M) on GABA-stimulated gastrin release. Antral gastrin secretion stimulated by 10^{-8} M GABA was inhibited by ~40% with the addition of 10^{-8} M bicuculline. Further inhibition of GABA-stimulated gastrin release resulted from higher concentrations of medium bicuculline and GABA-stimulated gastrin release was returned to control (non-stimulated) levels with 10^{-6} M bicuculline. Inhibition of somatostatin release by GABA was prevented completely by bicuculline at a medium concentration of 10^{-7} M. Bicuculline alone, in concentrations ranging from 10^{-9} to 10^{-6} M, had no significant effects on either basal gastrin release or basal somatostatin release.

We concluded from these results that GABA is a potent stimulant of antral gastrin release *in vitro*. In addition, GABA is capable of causing simultaneous and dose-dependent inhibition of basal somatostatin release from antral mucosal fragments. The inverse relationship between GABA-mediated stimulation of antral gastrin release and inhibition of basal somatostatin secretion is similar in some respects to the findings of Saffouri *et al.*¹⁸. These investigators demonstrated a reciprocal functional relationship between gastrin and somatostatin secretion from the isolated perfused rat stomach during infusion of the cholinergic agent, methacholine. It has been postulated that somatostatin exerts a tonic paracrine restraining influence over antral gastrin release and that when this paracrine hormone effect is diminished, then gastrin release is enhanced. The complexity of proposed gastrin-somatostatin interaction is suggested by results from these and other studies¹⁹⁻²³. As shown in Fig. 1, maximum stimulation of gastrin release occurred with 10^{-8} M GABA. On the other hand, peak inhibition of somatostatin release occurred at a medium concentration of 10^{-6} M GABA. The observation that 10^{-8} M GABA inhibited somatostatin release by approximately 40% of maximum and yet this same concentration of GABA was associated with

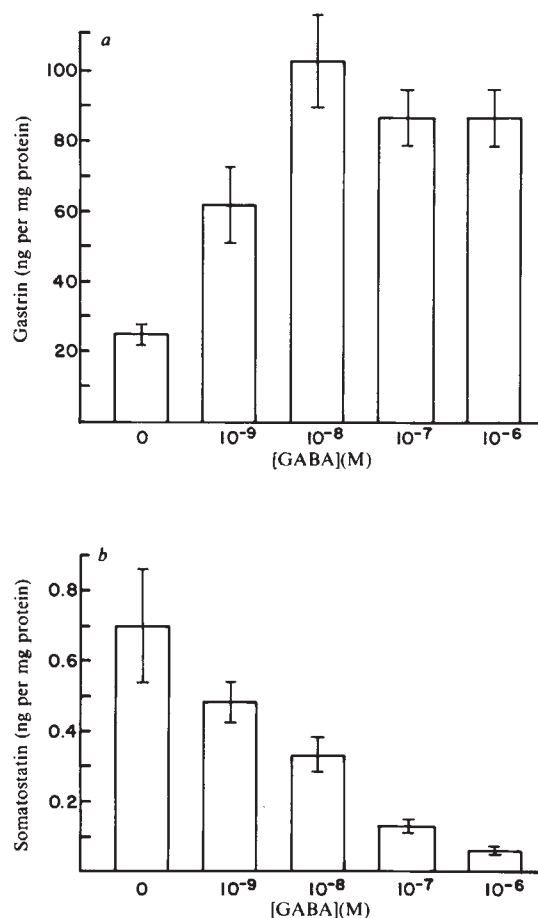


Fig. 1 Effect of increasing concentration of GABA in medium on release of gastrin (a) and somatostatin (b) from rat antral mucosal fragments during 1-h culture. Values are mean $\pm$ s.e.m. (bars) for triplicate determination of samples obtained from 10 individual cultures of antral mucosal fragments. Significant differences in gastrin and somatostatin release from control were obtained for each dose of GABA ($P < 0.05$).

Methods: Antral mucosa was obtained from fasted adult male Wistar rats (200–250 g) and sliced into 225 μ m sections on a Sorvall TC-2 tissue sectioner. The tissue was preincubated for 45 min stabilization in Krebs–Henseleit bicarbonate buffer, pH 7.4. Both stabilization and subsequent test incubations were performed at 37 °C in a Dubnoff metabolic shaker (100 oscillations per min) in the presence of 95% O₂, 5% CO₂. After stabilization the antral sections were aliquoted into 1-ml suspensions with each tube containing 0.1–0.5 mg antral protein. Antral mucosal fragments were incubated in either control or test media for 60 min. At the termination of test incubations (60 min) media were sampled for gastrin and gastrin contents were measured by radioimmunoassay²⁷. In addition, media somatostatin contents were determined by radioimmunoassay by the double antibody method. The primary antibody was rabbit antibody (GT117-3) to cyclic somatostatin tetradecapeptide which demonstrated equivalent immunological reactivity for somatostatin 28 and somatostatin 14. Somatostatin antibody demonstrated no immunological cross-reactivity with other gastrointestinal regulatory peptides, including gastrin, vasoactive intestinal peptide, cholecystokinin octapeptide, glucagon and secretin. The lower limit of somatostatin assay sensitivity was 8 pg ml⁻¹ or 0.8 pg per incubation tube. Recovery of gastrin and somatostatin added (10-fold excess above basal values) to the culture media and incubated for 60 min in the presence of antral tissue was 95.2 $\pm$ 1.4% and 93.7 $\pm$ 8.7%, respectively. Antral tissue gastrin and somatostatin were extracted by boiling tissue fragments in deionized water for 30 min. Residual tissue was preserved and protein content determined by the Lowry method¹⁶. Statistical significance was ascribed to values with $P < 0.05$ as determined by Student's *t*-test for unpaired data.

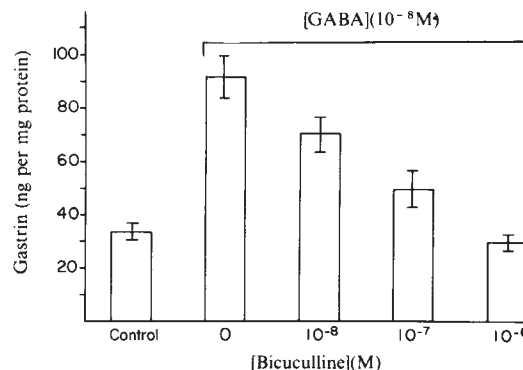


Fig. 2 Effects of GABA receptor blockade by bicuculline on GABA-stimulated antral gastrin release. The bars represent the mean $\pm$ s.e.m. of triplicate gastrin determinations for 10–20 replicate cultures of antral mucosal fragments. Bicuculline inhibited GABA-stimulated gastrin release in a dose-dependent manner.

maximal stimulation of antral gastrin release suggests that factors other than inhibition of antral somatostatin release may contribute to GABA-mediated stimulation of antral gastrin release. The present studies do not define the exact site of action of GABA in modifying antral peptide hormone secretion. It is possible that GABA exerts a direct effect on antral G and D cells to promote stimulation or inhibition of secretion of gastrin and somatostatin. Alternatively, GABA may act indirectly to influence antral peptide hormone release. In this regard, it is well known that GABA may act as a neural modulator in controlling release of other neurotransmitter substances such as the cholinergic agent acetylcholine^{24–26}. Although these studies do not define the exact site(s) of action of GABA, it is clear that bicuculline, a specific GABA receptor antagonist, is capable of preventing, in a dose-dependent manner, the effects of GABA on antral gastrin and somatostatin release.

Future studies will be needed to define more clearly and establish the anatomical and functional presence of GABAergic neurones in the proximity of mucosal endocrine cells of the stomach and proximal small intestine.

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Tolerance of T-cell clones is associated with membrane antigen changes

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It is possible to regulate the activity of human influenza virus specific helper T-cell clones either by high concentrations of antigen¹ or by anti-idiotypic suppressor T cells². In the absence of accessory cells, the appropriate peptide antigen recognized by the clones induces specific unresponsiveness. This phenomenon, however, is not the result of cytotoxicity as responsiveness to IL-2 remained unaltered. This suggests that high-dose immunological tolerance need not involve suppressor T cells, and that peptide antigens can interact directly with the T-cell surface. As recent reports suggest that the T-cell surface antigen T3 is involved in the triggering of T lymphocytes and possibly in antigen recognition^{3,4} we have investigated the expression of T3 and other cell surface antigens following the induction of T-cell tolerance. We report here that when a T-cell clone is exposed to a tolerizing concentration of the appropriate peptide antigen, surface T3 antigen is lost in a dose-dependent manner. As loss of surface T3 induced by anti-T3 antibody also results in unresponsiveness to antigen, we conclude that T3 is involved in the process of T-cell triggering by antigen.

The capacity to produce and maintain long-term T-lymphocyte clones has made it possible to analyse T-cell heterogeneity and function with much greater precision than before. We have generated a number of human influenza specific T-cell clones and lines which show diversity of function, such as help⁵ suppression² and lymphokine production⁶. Some of these have been analysed for the fine specificity of their response to antigen. Clone HA1.7 has been shown to proliferate in culture in response to HLA-DR1 and a 24 amino acid peptide derived from the C-terminal of the HA1 portion of the influenza haemagglutinin (residues 306–329; termed peptide 20)⁷.

We have shown that when clone HA1.7 was cultured for several hours at 37 °C with a supraoptimal dose of peptide 20 (p. 20) in the absence of antigen presenting cells, the clone failed to respond by proliferation when subsequently exposed to an immunogenic dose with antigen presenting cells. This unresponsiveness is a form of immunological tolerance and is antigen specific, since the HA1.7 cells which cannot respond to antigen still proliferate in response to optimal concentrations of TCGF. Other T-cell clones in the same culture vessel remain unaffected indicating that this is a specific effect. The cells remain unresponsive for at least one week but during this period are alive and capable of responding to TCGF. These results exclude certain hypotheses for the mechanism of tolerance¹.

Since the T3 antigen is associated with lymphocyte triggering its expression was investigated in clone HA1.7 cultured for 16 h at 37 °C in the absence of feeder cells, or with the relevant influenza A haemagglutinin peptide, p. 20, or an irrelevant haemagglutinin peptide, p. 11. The cells were examined for the expression of T3 antigen by indirect immunofluorescence using a fluorescence activated cell sorter (FACS IV, Becton Dickinson) and for their capacity to respond by proliferation with antigen (p. 20) in the presence of histocompatible irradiated antigen presenting cells (Fig. 1). When cells have been pre-incubated in medium or with p. 11, the majority of the cells

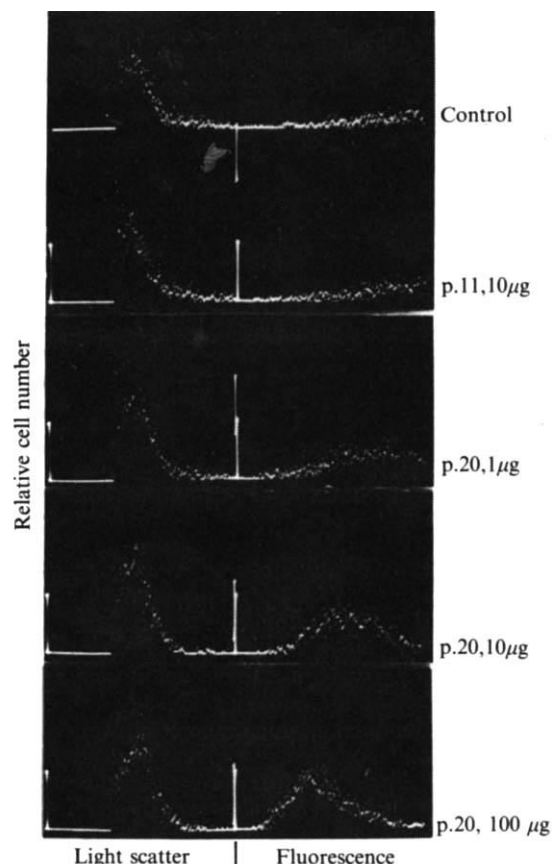


Fig. 1 Indirect immunofluorescent staining with UCHT1 monoclonal antibody of control and peptide antigen modulated clone HA1.7. T lymphocyte clones specific for the synthetic peptides of influenza haemagglutinin⁷ were isolated as previously described^{1,18}. Briefly, peripheral blood lymphocytes (PBL) were cultured for 6 days with $0.1 \mu\text{g ml}^{-1}$ of HA (gift of Dr R. G. Webster). The lymphoblasts were enriched on a discontinuous Percoll (Pharmacia) gradient and resuspended in RPMI-1640 (Gibco) containing 10% A + serum and 20% T-cell growth factor (TCGF), and plated at one cell every third well in Teraski trays with 10^4 irradiated (2,500 rad) autologous PBL and $0.1 \mu\text{g ml}^{-1}$ HA. After 7 d, growing clones were transferred to 96-well microtitre trays and then to 24-well trays. At each transfer the clones received fresh TCGF and irradiated autologous PBLs together with specific antigen. The clones were expanded in 25 cm^2 tissue culture flasks receiving 20% TCGF every 3–4 d and irradiated autologous PBL and intact virus (A/Texas/1/77) every 7 d. TCGF was prepared from 48 h supernatants of PBL (1×10^6 per ml) cultured with 0.1% purified phytohaemagglutinin (PHA-P; Difco) in complete culture medium containing 1% autologous serum. Before use in tolerance induction or modulation assays, the clones were rested for 6–7 d after the addition of filler cells. Modulation was induced by exposing 2×10^5 clone cells in round bottom microtitre trays in the absence of filler cells for 16 h to HA peptides at the concentrations shown in the figure. In these experiments the peptides used were p. 11 (amino acid residues 105–140) and p. 20 (residues 306–329). Following exposure to peptide or control medium the cells were washed and stained for indirect immunofluorescence with UCHT1 monoclonal antibody and FITC conjugated and immunoabsorbent purified sheep anti-mouse immunoglobulin antiserum¹⁰. Stained cells were analysed for forward angle light scattering (cell size) and scatter gated fluorescence on a FACS IV.

are brightly stained by UCHT1 (anti-T3) and far to the right of the scale, but after exposure to p. 20 the fluorescence is much reduced. A dose-response curve with p. 20 shows that the diminution of T3 is dependent on antigen dose, increasing up to the highest concentration used. The phenotypic effects observed following the exposure of HA1.7 to p. 20 are specific in that an irrelevant peptide (p. 11) does not induce the modulation of T3 (Fig. 1). Furthermore, the modulation was not a

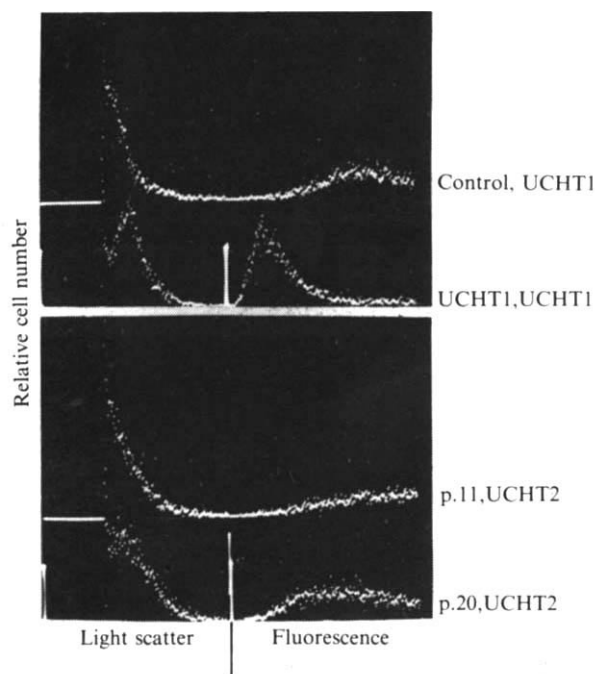


Fig. 2 Modulation of surface antigens by exposure to antibody or peptide antigen. Clone HA1.7 cells were cultured for 16 h in medium alone, in the presence of $5 \mu\text{g ml}^{-1}$ of purified UCHT1 immunoglobulin or with $20 \mu\text{g ml}^{-1}$ of p. 11 or p. 20 (see legend to Fig. 1). Following treatment cells were washed and stained with either UCHT1 (anti-T3) or UCHT2 (anti-T1) monoclonal antibodies followed by FITC sheep anti-mouse immunoglobulin anti-serum.

unique property of either clone HA1.7 or p. 20 in that clone HA2.43, also specific for p. 20, was modulated in a similar manner (Table 1). Secondly clone HA2.61, specific for p. 11 (ref. 1), showed modulation of T3 only after exposure to p. 11 and not p. 20 (Table 1). Exposure of clones HA1.7, HA2.43 and HA2.61 to UCHT1 antibody under similar conditions also leads to a loss of surface T3 antigen (Table 1, Fig. 2) alone, but not other surface markers studied. Preliminary data suggests that significant changes in phenotype and function can be detected after 3 to 4 hours exposure to antigen or UCHT1 antibody. We have not yet investigated in detail the kinetics of re-expression of T3 antigen on modulated cells but other data suggests that modulated TCGF dependent T cells re-express T3 after approximately 48 h⁶.

The functional effects of pretreatment with antigen or various antibodies are shown in Table 2. When clone HA1.7 is used, p. 20 but not p. 11 markedly inhibits the response to its specific antigen p. 20, but not to TCGF, indicating that it is an antigen specific effect. UCHT1 causes similar abrogation of the response to antigen. Of the other antibodies, anti-T1 (Leu 1, UCHT2) was weakly inhibitory but not any of the others. It may be relevant that the antigen T1 was partly modulated by p. 20 but not p. 11 (Fig. 2) and can be readily modulated by exposure to anti-T1 antibody (data not shown and reference 8). All the other antibodies shown in Table 2 stained HA1.7 but the level of staining was not decreased by pre-exposure to p. 20.

We have previously shown that exposure of T cell clones to a high dose of peptide antigen in the absence of accessory cells induces a state of specific immunological tolerance implying that T lymphocytes can recognise soluble antigen without processing¹. The results presented here lend support to this view as, in addition to the functional effects, we have shown that the cells undergo profound phenotypic changes. The data also imply that the loss of T3 antigen is intimately associated with the loss of responsiveness since both the phenotypic and functional changes can be induced by exposure to antigen or anti-T3 antibody and have the same antigen dosage requirements. It is

Table 1 Modulation of T3 antigen in control clones

Clone	Pre-incubation	% Of cells stained with UCHT1 in fluorescence channels		
		1-100	101-200	201-255
HA2.61 (anti-p. 11)	p. 11	29	31	40
	p. 20	4	13	83
	UCHT1	63	25	12
HA2.43 (anti-p. 20)	p. 11	8	11	81
	p. 20	18	25	57
	UCHT1	53	26	21

T-cell clone 2.61 responds by proliferation to p. 11 and T-cell clone 2.43 to p. 20. Both clones were incubated for 16 h at 37°C with $20 \mu\text{g ml}^{-1}$ of p. 11 or p. 20 or $5 \mu\text{g ml}^{-1}$ of UCHT1 monoclonal antibody. They were then washed and stained with UCHT1 monoclonal antibody for FACS analysis (see Fig. 1). Results are expressed as the percentage of cells (10^3) analysed appearing in fluorescence channels. Cells appearing in channels 1-100 are unstained or very dim, those in channels 101-200 are of intermediate brightness and those in channels 201-255 are brightly stained.

Table 2 Effect of monoclonal antibodies reactive with lymphocyte antigens on the induction of antigen specific unresponsiveness

Antibody (specificity)	Tolerance induction Antigen	Response (c.p.m. $\pm$ s.e.m.)	
		Antigen	TCGF
—	—	8,821 $\pm$ 585	3,981 $\pm$ 499
—	p. 20	270 $\pm$ 67	4,353 $\pm$ 479
UCHT1 (T3)	—	431 $\pm$ 70	4,597 $\pm$ 412
Leu 1 (T1)	—	6,209 $\pm$ 1,251	4,726 $\pm$ 442
UCHT1 (T1)	—	4,700 $\pm$ 567	4,233 $\pm$ 507
9.6 (E receptor)	—	8,271 $\pm$ 197	3,968 $\pm$ 390
3A1 (most T cells)	—	7,787 $\pm$ 1,026	4,593 $\pm$ 391
M-T321 (T4)	—	7,885 $\pm$ 731	3,951 $\pm$ 522
2A1 (HLA Class I)	—	8,995 $\pm$ 869	4,871 $\pm$ 254
M8 (β 2M)	—	9,275 $\pm$ 178	4,730 $\pm$ 497
Ra1a (HLA Class II)	—	7,291 $\pm$ 562	4,103 $\pm$ 451
HLA-1 (leukocyte common)	—	6,803 $\pm$ 522	4,182 $\pm$ 169
—	p. 11	7,984 $\pm$ 937	4,144 $\pm$ 536

Tolerance was induced as described in detail elsewhere¹. Cloned T cells (10^5 per ml) were incubated for 16 h with haemagglutinin (HA) peptides or antibodies in the absence of antigen presenting cells. HA peptides were used at $50 \mu\text{g ml}^{-1}$, the monoclonal antibodies UCHT1 and Leu 1 at $5 \mu\text{g ml}^{-1}$ of purified immunoglobulin and other monoclonal antibodies as whole ascites or serum at concentrations at least fivefold greater than the minimum required to give maximal staining by indirect immunofluorescence. After washing, the T cells (2.5×10^4 per ml) were added to irradiated autologous sheep erythrocyte rosette negative (E-) cells (2.5×10^4 per ml) that had been pulsed with antigen. Following 72 h incubation the cultures were pulsed for 8-16 h with $1.0 \mu\text{Ci } ^3\text{H TdR}$ (Radiochemicals Inc., Amersham) and collected on to glass fibre filters. Proliferation as correlated with $^3\text{HTdR}$ incorporation was measured by liquid scintillation spectroscopy. UCHT1 is specific for the T3 antigen present on all mature functional T cells¹⁰, UCHT2 and Leu 1 are against the 67K T1 antigen^{11,12}, 9.6 binds to the sheep red blood cell receptor¹³, 3A1 stains the majority of T cells¹⁴, M-T321 is against the helper/inducer subset (P. Rieber, personal communication), 2A1 is against a non-polymorphic determinant of HLA Class I¹⁵, M8 is against beta-2M¹⁶, anti-HLA-1 is against a common leukocyte antigen¹⁵ and the rabbit anti-Ia is broadly reactive with human Ia-like antigens¹⁷. HA1.7 expresses the phenotype characteristic of human helper cells (T1, 3, 4 and 11 positive) and is stained by all the reagents listed above.

conceivable, however, that HA1.7 recognizes antigen in association with its own Ia molecules, but fails to proliferate in the absence of other stimulating signals. This question can only be answered by direct binding studies using labelled antigen.

Our results resemble the recent observations of Schlossman and his colleagues who have reported that the T3 antigen of cloned human cytotoxic cells was modulated by exposure to anti-T3 or antibody to clonal specific (clonotypic) molecules⁴. Comodulation suggested that the T3 antigen was closely associated with the clonotypic marker which was considered to be the receptor for antigen. Taken together, both sets of experiments imply that T3 is associated with T-cell antigen recognition. However, certain differences are apparent that influence our hypothesis as to the functional role of T3 and its precise association with the antigen specific T-cell receptor. It has been suggested, based on the comodulation of phenotype and

function that T3 represents the constant region of the antigen recognition structure. However, it is unlikely that T3 by itself represents the entire constant region since serological evidence indicates differences between the constant region of the receptors of helper and suppressor T-cell subpopulations⁹.

The induction of unresponsiveness by anti-T3 or p. 20 resulted in no enhancement of the IL-2 response, implying that although T3 may be required for antigen-specific signal transmission it is not a prerequisite for IL-2 signal transmission. Furthermore, pretreatment with antigen (or anti-T1) induced phenotypic changes not seen with anti-T3, namely modulation of the T1 antigen. This suggests that although T3 may be linked to the antigen specific receptor it is unlikely that the two are identical since anti-T3 and specific antigen modulate T cells in a different manner. It is our view therefore that T3 is not a structural component of the T-cell receptor but is necessary for transmission of an activation signal following antigen binding. This hypothesis is compatible with all the reported immunological effects of anti-T3.

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Immunization to a syngeneic sarcoma by a monoclonal auto-anti-idiotypic antibody

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Idiotypic networks regulate the immune response to a variety of antigens¹⁻³. Antibodies generated against other antibodies, called anti-idiotypic antibodies, can themselves mimic antigen and elicit a specific immune response. They have been shown to induce delayed-type hypersensitivity (DTH) to model antigens in the mouse⁴⁻⁷. As anti-idiotypic antibodies are thought to be involved in the response to tumour-associated antigens⁸⁻¹⁰ we tested whether injection of monoclonal antibodies derived from mice hyperimmunized to a syngeneic, chemically induced sarcoma could mimic antigen and induce DTH to the sarcoma in naive mice. One of the monoclonal antibodies, 4.72, primed BALB/c mice for DTH to the sarcoma but not for DTH to another sarcoma or to sheep erythrocytes. Antibody 4.72 did

not induce DTH in mice of immunoglobulin allotype congenic strains nor did it bind to the sarcoma cells. As antibodies specific for this sarcoma have not been detected, we do not know whether idiotype on immunoglobulin molecules is recognized by antibody 4.72. However, as the response induced by antibody 4.72 was both antigen-specific and allotype-restricted, analogous to those induced by anti-idiotypic antibodies in other systems^{4,11}, we propose that antibody 4.72 is an anti-idiotypic antibody.

Anti-idiotypic antibodies characteristically regulate the immune response by virtue of their reactivity with specific determinants known as idiotypes on B and T lymphocytes. They lack antigen binding activity, and they may alter the clonal profile of the immune response by stimulating or suppressing antigen-specific immunity in an immunoglobulin heavy chain (Igh)-restricted manner^{1-4,12,13}. To test the hypothesis that idiotype interactions influence the immune response to tumour-associated antigens, we looked for evidence of auto-anti-idiotypic antibodies in tumour-immune mice. Spleen cells from a mouse immunized to the sarcoma MCA-1490, by excision of a transplanted tumour, followed by two injections of tumour cells, were fused with the non-secreting myeloma, P3-NS1-1-Ag4-1 (NS-1)¹⁴ (Table 1). In initial screening of hybridoma supernatants, one hybridoma (4.72) produced an antibody (IgG1) that, after injection subcutaneously into naive BALB/c mice, primed them for DTH to MCA-1490. This hybridoma was cloned and expanded in culture. We found that 5 µg of antibody 4.72 per mouse primed for DTH reactivity to MCA-1490 but not for DTH to an antigenically different sarcoma,

Table 1 Monoclonal antibody 4.72 induced DTH to MCA-1490

Mice immunized with	Mean % footpad swelling (±s.e.) of mice challenged with:		
	MCA-1490	MCA-1511	SRBC
Diluent	5.8±0.7	5.6±1.1	7.4±2.0
Antibody 8.2	5.4±1.0	3.6±1.1	5.4±1.3
Antibody 4.72	19.2±1.4*	3.2±1.1	5.6±0.9
MCA-1490 cells	24.8±2.3*	ND	ND
MCA-1511 cells	ND	27.0±2.7*	ND
SRBC	ND	ND	34.8±2.7†
Fab 8.2	6.0±0.9	5.1±0.9	ND
Fab 4.72	20.3±1.7*	5.7±0.7	ND

To induce DTH, BALB/c mice (five mice per group) were immunized by subcutaneous injection of 100 µl phosphate-buffered saline (PBS) containing 10⁶ tumour cells, 5.0 µg of monoclonal antibody or 3.0 µg of Fab fragments or by an intraperitoneal injection of 3×10⁷ SRBC in 100 µl PBS. DTH was elicited 5 days after priming with a challenge injection of 5×10⁵ tumour cells or 10⁸ SRBC in 20 µl PBS into one hind footpad. Footpad swelling was measured 24 h later and is presented as the mean per cent increase of the injected pads compared with the non-injected contralateral pads. Hybridoma 4.72 was cloned from a fusion of spleen cells from a MCA-1490-hyperimmunized BALB/c mouse and NS-1 myeloma cells¹⁴. MCA-1490 and MCA-1511 were transplanted for less than 10 passages, and were tested as negative for mycoplasma and several passenger viruses. The fusion technique and growth of hybridoma in selective medium are described elsewhere¹⁵. After excision of a transplanted MCA-1490 tumour, mice were immunized twice at 2-week intervals with irradiated MCA-1490 cells²¹. Spleen cells were taken from the mice 7 days after the last boost with MCA-1490 cells and fused in a 4:1 ratio with NS-1 cells¹⁵. Ten days after the fusion, 400 hybridomas were tested for production of IgG antibody by a radioimmunoassay. Fourteen hybrids selected for IgG antibody production and vigorous growth were then expanded and cultured to obtain 2 ml of culture fluid which was tested directly by priming mice with 0.4 ml medium per mouse to elicit DTH to MCA-1490. One active hybrid (4.72) was cloned and expanded. Antibody 4.72 was purified from culture media by affinity chromatography on *Staphylococcus aureus* protein A coupled to Sepharose CL-4B (ref. 24). The production and specificity of monoclonal antibody 8.2 are described elsewhere¹⁵. Fab fragments from antibody 4.72 and antibody 8.2 were prepared by papain digestion as described previously²⁵. ND, not done.

* $P < 0.001$ or † $P < 0.0001$ by Student's *t*-test compared to group immunized with diluent alone.

Table 2 Antibody 4.72 induced lymphoid cells which expressed DTH to MCA-1490 in naive recipients

Donor mice immunized with	Mean % footpad swelling ($\pm$ s.e.) of recipients of lymphoid cells and	
	MCA-1490	MCA-1511
Antibody 8.2	3.0 $\pm$ 1.9	4.7 $\pm$ 1.3
Antibody 4.72	15.7 $\pm$ 0.3*	7.0 $\pm$ 1.9
MCA-1490 cells	20.0 $\pm$ 4.3*	ND
MCA-1511 cells	ND	20.0 $\pm$ 1.0†

Donor BALB/c mice were immunized with antibody or tumour cells as described in Table 1. After 5 days, they were injected intraperitoneally with 10 mg of casein in 2 ml of PBS: peritoneal exudates were collected 24 h later. Lymphoid cells were obtained by removal of adherent cells on Sephadex G-10, were mixed with tumour cells and were injected into one hind footpad of each of five naive BALB/c mice. Each mouse received 10^6 lymphoid cells and 5×10^5 tumour cells. Footpad swelling was measured 24 h later. ND, not done.

*† This response was greater than that of mice receiving tumour cells and lymphoid cells from mice primed with antibody 8.2; * $P < 0.01$ or † $P < 0.001$ by Student's *t*-test.

MCA-1511, or to SRBC (sheep red blood cells; Table 1). This amount of antibody is similar to that required to prime for DTH in the azobenzene-arsonate system⁶. Mice primed with control monoclonal IgG1 antibody, 8.2 (ref. 15), specific for a human melanoma-associated antigen, were not immunized to MCA-1490, MCA-1511 or SRBC. Fab fragments (3 μ g) derived from antibody 4.72 but not those of 8.2 also specifically immunized BALB/c mice to MCA-1490 (Table 1). This experiment has been repeated twice in its entirety and in part up to 20 times with similar results. The response induced by antibody 4.72 was shown to be mediated by lymphoid cells using adoptive transfer to naive recipients (Table 2). Lymphoid cells from BALB/c mice primed with antibody 4.72, antibody 8.2, or tumour cells were mixed with either MCA-1490 cells or MCA-1511 cells and injected into the footpads of syngeneic mice. Priming with antibody 4.72 induced lymphoid cells which transferred DTH to MCA-1490 but not to MCA-1511. Priming with either tumour generated lymphoid cells which transferred DTH to that tumour.

We next studied whether ¹²⁵I-labelled¹⁶ antibody 4.72 or Fab 4.72 could bind to MCA-1490 cells. Binding was not detected, indicating that 4.72 does not recognize a tumour-associated membrane determinant (data not shown).

As induction of cellular immune responses by anti-idiotypic antibody has been shown to require homology at the *Igh-1* locus between host and antibody^{13,17}, we assayed whether antibody 4.72 induced DTH to MCA-1490 in the allotype congenic strains BALB/c (*Igh-1*^a), CB-20 (*Igh-1*^b) and CAL-20 (*Igh-1*^d). Table 3 presents one of two experiments showing that antibody 4.72 primed BALB/c mice but not CB-20 mice or CAL-20 mice for DTH to MCA-1490. Mice from all three of these strains responded to priming with MCA-1490 cells and none of the mice responded after priming with antibody 8.2. These data suggest that antibody 4.72 induced idiotype-positive cells which mediated DTH to MCA-1490. Therefore, we conclude that antibody 4.72 is a member of the *Igh-1*^a-linked repertoire of idiotypes and anti-idiotypes that respond to a determinant on MCA-1490. As 4.72 does not bind to MCA-1490, it is likely that the antibody is anti-idiotypic to the receptors of cells responding to MCA-1490. Similar observations in an alloimmune system have been described by Bluestone *et al.*¹¹. By immunizing mice with anti-idiotypic antibodies specific for monoclonal anti-H-2^k antibodies, they observed allotype-linked induction of idiotype positive molecules.

Our results with antibody 4.72 could be reproduced using antiserum from BALB/c mice hyperimmunized to either MCA-1490 or MCA-1511. As shown in Table 4, injection of 100 μ g of IgG from mice hyperimmunized to MCA-1490 primed BALB/c mice for DTH to MCA-1490 but not to MCA-1511. IgG from mice hyperimmunized to MCA-1511 induced DTH

Table 3 Antibody 4.72 did not induce DTH to MCA-1490 in allotype congenic mice

Mice immunized with	Mean % footpad swelling ($\pm$ s.e.)		
	BALB/c	CB-20	CAL-20
Antibody 8.2	6.7 $\pm$ 1.1	8.0 $\pm$ 0.4	7.0 $\pm$ 0.4
Antibody 4.72	22.3 $\pm$ 1.4*	8.3 $\pm$ 0.9	11.7 $\pm$ 0.9
MCA-1490 cells	24.3 $\pm$ 1.4*	20.0 $\pm$ 0.8†	19.3 $\pm$ 0.7†

Mice were immunized with purified antibody (5 μ g) and DTH elicited and measured as described in Table 1. Breeding pairs of CB-20 (ref. 26) and CAL-20 (ref. 27) were obtained from Dr M. Potter, NCI contract NO1-CB-25584. All mice were bred and maintained in the Fred Hutchinson Cancer Research Center Animal Facility.

*† Response significantly different from the group immunized with antibody 8.2; * $P < 0.001$ or † $P < 0.01$ by Student's *t*-test.

Table 4 IgG from the sera of mice hyperimmunized to either MCA-1490 or MCA-1511 induced DTH to the immunizing tumour

Mice immunized with	Mean % footpad swelling ($\pm$ s.e.) of mice challenged with	
	MCA-1490	MCA-1511
Diluent	6.0 $\pm$ 0.5	6.3 $\pm$ 2.5
Control IgG	5.6 $\pm$ 0.7	4.0 $\pm$ 1.0
Anti-MCA-1490 IgG	22.2 $\pm$ 2.1*	4.0 $\pm$ 1.5
Anti-MCA-1511 IgG	11.4 $\pm$ 2.8†	25.2 $\pm$ 2.7†
MCA-1490 cells	36.0 $\pm$ 2.0*	16.6 $\pm$ 3.5
MCA-1511 cells	10.0 $\pm$ 2.5	37.2 $\pm$ 3.8‡

Mice were immunized with IgG (100 μ g) or tumour cells and DTH elicited and measured as described in Table 1. BALB/c mice were hyperimmunized to MCA-1490 or MCA-1511 as described in Table 1. The IgG fractions of the sera of hyperimmunized mice and control mice were obtained by affinity chromatography on *S. aureus* protein A coupled to Sepharose CL-4B (ref. 24).

*†‡ Response greater ($P < 0.001$, < 0.05 or < 0.01 , respectively, by Student's *t*-test) than that of the group immunized with diluent.

to MCA-1511 and a low but significant response to MCA-1490. DTH to either tumour was not induced by priming with IgG from mice not immunized with tumour cells. In experiments on idiotype regulation in alloimmunity, McKearn *et al.*¹⁸ have also documented that auto-anti-idiotypic antibodies arise after repeated immunization using a protocol similar to ours.

We postulate that antibody 4.72 is anti-idiotypic for the following reasons. Immunization with antibody 4.72 primes for a response which seems to be specific for an antigen on MCA-1490 cells. As this immunization also appears to be restricted to allotype-compatible combinations, it maps the phenomenon to *Igh-1*-linked genes; this is characteristic of cellular idiotype networks⁴. Finally, serum antibodies with activity which mimics that of antibody 4.72 arise after repeated immunization with MCA-1490 cells; this is consistent with the hypothesis that 4.72 is representative of a naturally occurring antibody. In model antigen systems, such anti-idiotypic antibodies also bind idiotype immunoglobulin with a high degree of specificity. We could not carry out this assay as we have been unable to detect antibodies specific for unique tumour antigens in our tumour-immune mice¹⁹. Thus, we have no immunoglobulin idiotype to correspond to the anti-idiotypic activity. This implies that antibody 4.72, or other similar putative anti-idiotypic antibodies, may be generated in response to cellular idiotypes present on lymphocytes during the immune response to tumour. We are now looking for evidence of such complementary 'idiotype' lymphocyte populations among effector and suppressor subsets of tumour-immune cells and hybridomas²⁰.

Idiotypic interaction in the regulation of tumour immunity has been suggested by the observations that 'unblocking' antibodies from mice immunized to a syngeneic, chemically-induced sarcoma bound specifically to tumour antigen-specific suppressor 'blocking' factors from mice carrying the same sarcoma^{4,21,22}. Further evidence for idiotype-anti-idiotypic interactions in tumour immunity has come from studies in which

putative anti-idiotypic antibodies down-regulated tumour-immune T cells from rats²³ and mice⁹. The fact that hyper-immune sera induce tumour antigen-specific DTH suggests that the findings reported here are applicable to other tumour systems. Experiments in progress will determine whether 4.72 primes for DTH to an antigen restricted to MCA-1490 or expressed on other sarcomas, whether it identifies lymphocytes responding to MCA-1490, and whether it can be used to manipulate the immune response toward rejection of MCA-1490.

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Effect of haematopoietic cell growth factor on intracellular ATP levels

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Growth and development of haematopoietic cells *in vitro* require the presence of specific regulatory molecules. Some of these molecular species appear to have a broad specificity, being able to promote the proliferation and differentiation of multipotential cells, as well as megakaryocytic, erythroid and granulocytic-progenitor cells¹⁻³. Such factors are present in medium conditioned by the growth of lectin-stimulated mouse spleen cells or WEHI-3 myelomonocytic leukaemia cells (WEHI-CM)¹⁻⁵. Using WEHI-CM, we⁶ and others⁷⁻⁹ have been able to obtain permanently growing, non-leukaemic cell lines of a granulocytic or mast cell nature. Significantly, we have found that the factor in WEHI-CM necessary for the growth of these cells has co-purified with the multi-lineage stimulating activity present in WEHI-CM, suggesting that one molecule may be concerned in the development of multiple cell types³. We have now used these cells to investigate the mode of action of this haematopoietic cell growth factor and have found that the requirement of this factor for survival and growth may lie in its ability to modulate ATP levels within the cells.

The origin, isolation and phenotypes of these factor-dependent cells (FDC-P) have previously been reported^{6,10}. Briefly, FDC-P2 cells were derived from a long-term marrow culture of DBA/2 origin. The cells have a granulocytic morphology, express Fc receptors, Mac-1 (a 'macrophage specific' antigen) and are positive for chloroacetate esterase and nonspecific esterase activity. Medium conditioned by the growth of WEHI-3 myelomonocytic leukaemic cells was used as a source of haematopoietic growth factor (HCGF). In most experiments, a partially purified but highly enriched preparation of HCGF was used following a three-step purification based on hydrophobic Hchromatography on phenyl Sepharose, ammonium sulphate precipitation and gel filtration³.

Attempts to replace HCGF with other defined growth factors, mitogenic and proliferative agents proved unsuccessful in maintaining viability of the FDC-P cells (Table 1). These agents included epithelial and fibroblast growth factors as well as putative haematopoietic regulatory molecules and 'nonspecific' agents such as the corticosteroid hormones. Also, as cyclic AMP level modulation has previously been implicated in the regulation of haematopoietic cell proliferation and differentiation^{11,12}, we attempted to replace HCGF with agents which modulate cyclic AMP levels. Again, none of these maintained FDC-P2 cells in culture in the absence of HCGF.

We did, however, find that HCGF could be replaced by an ATP generating system composed of ATP, creatine phosphate and creatine phosphokinase (Fig. 1). In the absence of HCGF cell viability was completely lost within 48 h. In the presence of HCGF or an ATP regenerating system cell viability and total cell numbers were maintained.

We next determined which component(s) of the ATP regenerating system was necessary for maintenance of cell survival in the absence of HCGF. We found that ATP alone was capable of maintaining a significant level of viability but that creatine phosphate or creatine phosphokinase, individually or in combination did not maintain the cells. Furthermore, another ATP generating system consisting of ATP, phosphoenolpyruvate and pyruvate kinase was also capable of maintaining cell viability over a 48 h period. Thus, these data indicate that the essential component for the maintenance of cell viability in FDC-P2 cells in the absence of HCGF is ATP. Further evidence that this is the case was the demonstration that ADP, AMP, adenosine or the ATP analogue adenosine (β , γ imino) triphosphate (AppNHp) were not able to maintain cell viability (Fig. 1). Similar experiments on cultures freshly explanted from bone marrow have not, as yet, shown any ability of the ATP regenerating system to maintain CFU-S in culture.

That the proliferative integrity of the FDC-P2 cells is maintained by the ATP regenerating system was demonstrated by our observation that at any time point (at least up to 48 h) after HCGF replacement with an ATP regenerating system the cells could be subcultured back into HCGF with no loss of proliferative potential (results not shown). After 10-14 h culture in the absence of the HCGF and the ATP regenerating system respectively FDC-P cells were difficult to recover and after 24 h, cells would not subsequently recover when the growth factor was added to their medium (data not shown).

We next showed that intracellular ATP levels were maintained when the FDC-P2 cells were cultured in the presence of HCGF (Fig. 2) and that in the absence of growth factor the intracellular ATP levels fall dramatically (Fig. 2). This fall occurred steadily and was apparent after only 1½ h culture in the absence of HCGF. This observation suggests that through some mechanism this growth factor modulates ATP levels in FDC-P2 cells. In support of this we have demonstrated that the intracellular ATP levels of FDC-P2 cells are maintained when an extracellular ATP regenerating system is added to the HCGF-free medium (Fig. 2). Also it seems (Table 2) that this extracellular ATP enters the cells and can be used for the manufacture of macromolecular materials precipitable by perchloric acid (PCA). Thus, from our evidence we infer that the ability of HCGF to allow FDC-P2 cells to survive and prolifer-

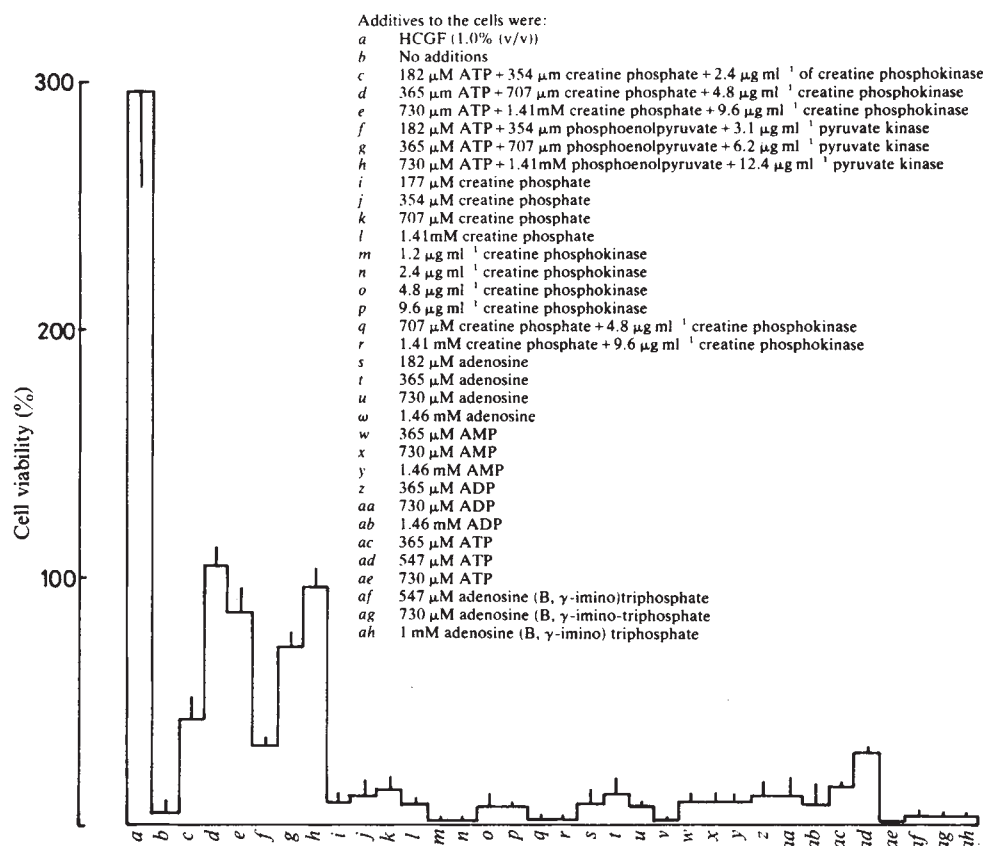


Fig. 1 FDC-P cells were maintained as in Table 1 legend. Viability was determined by trypan blue exclusion and the percentage of viable cells with respect to the number originally seeded at the beginning of the experiment determined after 48 h. Results are expressed as this percentage $\pm$ s.e.m. Experiments were performed at least twice and in the case of ATP (Boehringer) plus creatine phosphate (Sigma) plus creatine phosphokinase (Sigma) and ATP alone, HCGF alone, and in the absence of any additive at least six times. Adenosine and ADP were also from Sigma and all other additives were from Boehringer.

Table 1 The effect of growth factors and other agents on FDC-P2 cell viability

Additions	Concentration (range)	Viable cell no.	
		Inoculated	After 48 h culture
None		10^5	$<10^4$
HCGF	10^{-1} to 10^{-5} dilution (v/v)	10^5	$\sim 2.8 \times 10^5$
EGF	10 – 200 ng ml ⁻¹	10^5	$<10^4$
FGF	1 – 40 ng ml ⁻¹	10^5	$<10^4$
Hydrocortisone	10^{-5} – 10^{-10} M	10^5	$<10^4$
Testosterone	10^{-4} – 10^{-10} M	10^5	$<10^4$
Lactoferrin	10^{-8} – 10^{-16} M	10^5	$<10^4$
Prostaglandin E ₁	10^{-5} – 10^{-11} M	10^5	$<10^4$
GM-CSF (CSF-2)	10 – 1000 units per ml	10^5	$<10^4$
M-CSF (CSF-1)	10 – 1000 units per ml	10^5	$<10^4$
'CFU-S stimulator'	1 – 100 μ g ml ⁻¹	10^5	$<10^4$
Insulin	1 – 40 μ g ml ⁻¹	10^5	$<10^4$
Isobutylmethylxanthine	0.01 – 100 μ g ml ⁻¹	10^5	$<10^4$
Theophylline	5×10^{-3} – 10^{-7} M	10^5	$<10^4$
Cholera toxin	5×10^{-3} – 50 μ g ml ⁻¹	10^5	$<10^4$
Dibutyryl cyclic AMP	10^{-2} – 10^{-16} M	10^5	$<10^4$
Interleukin-2	10^{-1} – 10^{-4} dilution (v/v)	10^5	$<10^4$

FDC-P2 cells were routinely maintained in Fischer's medium (Gibco) supplemented with 20% (v/v) horse serum (Flow) and antibiotics as previously described⁶. WEHI-CM was prepared as previously and added to the culture as a source of HCGF at a final concentration of 10% (v/v). The cells were subcultured twice weekly by dilution into fresh growth medium to give between 5×10^4 and 1×10^5 cells per ml. For experimental use, the FDC-P2 cells were centrifuged (800g, 10 min), the supernatant was discarded and the cell pellet resuspended in Fischer's medium in the absence of HCGF. This was repeated twice to remove residual HCGF. After the second wash, the cells were resuspended in Fischer's medium to give a cell concentration of $\sim 1 \times 10^6$ cells ml⁻¹. 0.1 ml of the cell suspension was then added to 0.9 ml of Fischer's medium supplemented with 20% horse serum in 24-well Costar tissue culture plates, with or without HCGF or in the presence of the above additives. The plates were placed in a fully humidified incubator at 37°C. At times, thereafter, plates were removed, total cell counts performed, and the number of viable cells estimated by trypan blue exclusion. The HCGF used was a HPLC fraction with an activity of 10^6 units per ml (ref. 3). Fibroblast growth factor (FGF), epidermal growth factor (EGF), were purchased from Collaborative Research, cholera toxin, prostaglandin E₁, hydrocortisone, testosterone, theophylline, dibutyryl cyclic AMP and lactoferrin were from Sigma. Isobutylmethylxanthine was from Aldrich. The 'CFU-S stimulator' was supplied by Dr B. I. Lord, Paterson Labs, Manchester, as an Amicon ultrafiltrate which was active at a concentration of 2μ g ml⁻¹ on stimulating DNA synthesis in CFU-S¹⁵. GM-CSF (CSF-2) (granulocyte-macrophage colony stimulating factor) was supplied by Dr A. W. Burgess, Melbourne, Australia, and was a highly purified (step IV) preparation of mouse lung cell GM-CSF¹⁶ with approximate activity of 5,000 units per ml. M-CSF (CSF-1) macrophage-colony stimulating factor was supplied by Dr R. K. Shaduck, Pittsburgh, USA, as a highly purified preparation of mouse L-cell CSF¹⁷ with an approximate activity of 10,000 units per ml. Interleukin-2 was a purified preparation from mouse lymphocytes supplied by Dr S. Gillis, Seattle, USA. This preparation was active in stimulating the growth of murine T cells when used at a final concentration of 1%.

ate must be related to its apparent ability to maintain ATP levels within the cell. Furthermore, when FDC-P2 cells were cultured in the absence of HCGF for 5 h, then supplemented with HCGF, intracellular ATP levels increased within 10 min (Fig. 2). The short time interval would argue that the ability of HCGF to modulate cell ATP levels is of major importance in the maintenance of a viable state. However, we cannot exclude the possibility that HCGF primarily acts to allow the cell to retain intracellular metabolites (including ATP) and that its absence provokes extensive leakage, although no ATP could be found in the extracellular medium of cells cultured in the absence of HCGF.

In conclusion, it seems that HCGF, which is necessary for the survival and growth of FDC-P cells, is able to modulate ATP levels and that HCGF can be replaced by the addition of an ATP regenerating system to the cells. It appears that HCGF is a multi-lineage stimulating factor^{2,3} that enhances self-renewal of multipotential and committed progenitor cells as well as promoting differentiation and development in the various myeloid lineages. Whether the effect we have observed

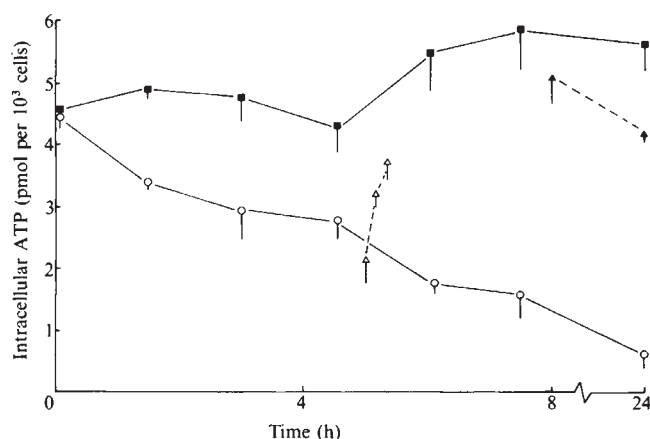


Fig. 2 Effect of HCGF on intracellular ATP levels of FDC-P cells. Experiments performed were FDC-P2 in the presence (■) and absence (○) of HCGF and also in the absence of HCGF but with an ATP regenerating system consisting of 365 μ M ATP, 707 μ M creatine phosphate and 4.8 μ g ml⁻¹ creatine phosphokinase added (△). FDC-P2 cells were maintained as for Table 1. For experimental use FDC-P2 cells were centrifuged and washed twice in Fischer's medium. Cultures were set up in tissue culture flasks (25 cm² growing area, Sterilin) with 1.5×10^6 cells contained in 10 ml of growth medium with or without WEHI-CM as a source of HCGF. The cultures were maintained at 37°C in an atmosphere of air + 5% CO₂. After set incubation periods the cells were plunged into iced water and cooled to 4°C, subjected to centrifugation at 800g for 10 min and the supernatant carefully removed. No ATP was found in this supernatant fraction. The cells were resuspended in phosphate buffered saline and 50 μ l of cold PCA (20% w/v) added. After 5 min the loose PCA precipitate was vigorously resuspended and then centrifuged at 4,000g for 5 min. The supernatant was decanted and the PCA neutralized with a set volume of triethanolamine (0.5 M) and KOH (2 M). This solution was centrifuged again as above and the supernatant stored at -20°C before assay of ATP, using the luciferase reaction¹⁸. When an ATP regenerating system was present in the cell medium, two further washings of the cells were performed. To determine the effect of readdition of HCGF on cellular ATP levels (△) FDC-P2 (5×10^5 cells) were prepared as above, but were then incubated for 5 h at 37°C in medium free of both HCGF and the ATP regenerating system, washed as described in the legend to Fig. 1 legend and then resuspended to a volume of 0.5 ml of Fischer's medium. After a preincubation period of 15 min at 37°C the purified HCGF was added to the cells and cold PCA (20% w/v, 50 μ l) added after 0, 5 and 10 min to lyse the cells. Immediately after this addition the cells were vigorously vortexed, plunged into iced water and prepared for ATP assay as described above. Results are expressed as the amount of ATP present per 10³ viable cells, viability being assessed as in Table 1 legend, the mean $\pm$ s.d. of at least three experiments is shown.

Table 2 Incorporation of ¹⁴C-ATP into FDC-P2 cells

Incubation time (h)	Incorporation of ¹⁴ C label into PCA precipitable material (% of total ¹⁴ C counts)	Incorporation of ¹⁴ C into PCA soluble material inside the cell (% of total ¹⁴ C counts)	¹⁴ C ATP in PCA soluble material (%)
0	0.008	0.018	ND
8	0.064	0.12	53
24	0.13	0.31	48

Cells (3×10^6) were incubated in the absence of HCGF with an ATP regenerating system described as in Fig. 2, except 0.15 μ Ci of ¹⁴C-ATP (Amersham International, 1.0 mCi mmol⁻¹) was also included. At set time intervals the cells were washed such that <0.001% of the counts associated with the medium were left in the final washing and the cells were then disrupted as described in Fig. 2 legend. The PCA precipitable material was collected on Whatman GF/C glass fibre filters, washed five times with ice cold PCA (10% w/v) and then taken for liquid scintillation counting. The PCA soluble material was also collected and the ¹⁴C activity determined, and expressed as a percentage of the exogenous ¹⁴C activity added to the cells. Samples of the PCA soluble intracellular material were taken for TLC analysis using an isobutyric acid:NH₄OH:water (66:1:33) solvent system to determine the amount of ¹⁴C-ATP inside the cells. The ¹⁴C activity which ran with the standard ATP applied to the plate, at the same R_F value as previously determined for ATP in this solvent system was said to represent ¹⁴C-ATP. The amount of ¹⁴C-ATP is expressed above as a % of the total counts recovered from the thin layer plate. Results are the mean of two experiments. ND, not determined.

on the FDC-P2 cells has any bearing on the role of HCGF in these other, normal cell populations remains to be determined. However, it is a consistent finding that in the absence of the appropriate lineage restricted regulatory molecule, such as GM-CSF or erythropoietin, progenitor cells are lost from culture within a matter of hours. Furthermore, the importance of these growth factors in leukaemogenesis has yet to be established, but it is probable that the process of leukaemogenesis represents a loosening in the normal control mechanisms for growth and differentiation^{13,14}. It is tempting to speculate that haematopoietic cell growth and differentiation factors facilitate survival and development of the haematopoietic progenitor cells via an effect on energy metabolism and that in leukaemia this requirement has, in some way, been bypassed.

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Red cell membrane abnormalities in chronic myeloid leukaemia

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Chronic myeloid leukaemia (CML) is a clonal neoplasm that arises in a stem cell common to granulocytes and erythrocytes¹⁻³. Several abnormalities have been identified in the plasma membranes of granulocytes of CML patients⁴⁻⁶, but to our knowledge no studies have been done on CML erythrocytes. We report here that CML erythrocyte spectrin becomes abnormal due to cross-linking of its two subunits via disulphide bonds. In addition, we show that this cytoskeletal defect in the erythrocytes is associated with loss of transmembrane phospholipid asymmetry. These observations, apart from demonstrating membrane abnormalities in CML erythrocytes, also provide strong support for the view that the asymmetric organization of phospholipids in the red cell membrane is maintained mainly by interactions between spectrin and aminophospholipids⁷⁻¹⁰.

Blood from leukaemic male patients (aged 30-50 yr) and from healthy adult donors was obtained from King George's Medical College, Lucknow, and drawn by venepuncture into heparinized glass tubes. All the patients studied here were established cases of CML as shown by their haematological and clinical analyses. After removal of plasma from blood cells, the cells were washed three times with phosphate-buffered saline (PBS, pH 7.2). Leukocytes, platelets and immature cells were separated from erythrocytes by using a Ficoll-Hypaque gradient¹¹. Red cells, obtained after the second gradient, were contaminated with <0.2% leukocytes and immature cells.

Membranes of CML and normal human erythrocytes were analysed for their protein compositions by SDS-polyacrylamide gel electrophoresis¹² in non-reducing conditions. The gels were stained for protein with Coomassie blue and scanned at 530 nm. Figure 1 shows typical densitometer scans of the gels. The gel profiles of CML erythrocyte ghosts contained one additional high-molecular weight protein band (H_p , Fig. 1C) which was absent from the gel patterns of normal red cell ghosts (Fig. 1A). Apart from this difference between the two types of cells, the CML erythrocyte membrane also contained smaller amounts of spectrin (Fig. 1C). Treatment of CML erythrocyte ghosts with β -mercaptoethanol (β -ME) at 37 °C for 15 min before their extraction resulted in the disappearance of the H_p band and consequently an increase in the intensities of spectrin bands (Fig. 1D). No effect on the gel profiles of normal erythrocyte ghosts was observed after treatment with β -ME in identical conditions (Fig. 1B). These findings clearly indicate that the H_p protein in CML erythrocyte membrane has originated from cross-linking of spectrin via disulphide bonds. This is further supported by our observation that the glutathione (GSH) levels in CML erythrocytes were considerably lower than in normal cells (Table 1).

Similar spectrin oligomers have recently been observed in some types of glucose-6-phosphate dehydrogenase-deficient red cells¹³. This abnormality was found to be associated with other membrane changes such as increased bulk lipid fluidity and decreased cholesterol:phospholipid ratio. Cross-linked spectrin has also been detected in red cells after treatment with sulphhydryl oxidizing agents^{7,14-19}. These agents, in controlled conditions, exclusively cross-linked spectrin via disulphide bonds, which in turn has been shown to be associated with a decrease in erythrocyte deformability¹⁵, inhibition of diskocyte-echinocyte shape changes¹⁶, and reduced lateral diffusion of band 3 polypeptides¹⁷. Prolonged treatment of red cells with these oxidizing agents, however, resulted in extensive cross-linking of membrane proteins and consequently aggregation of

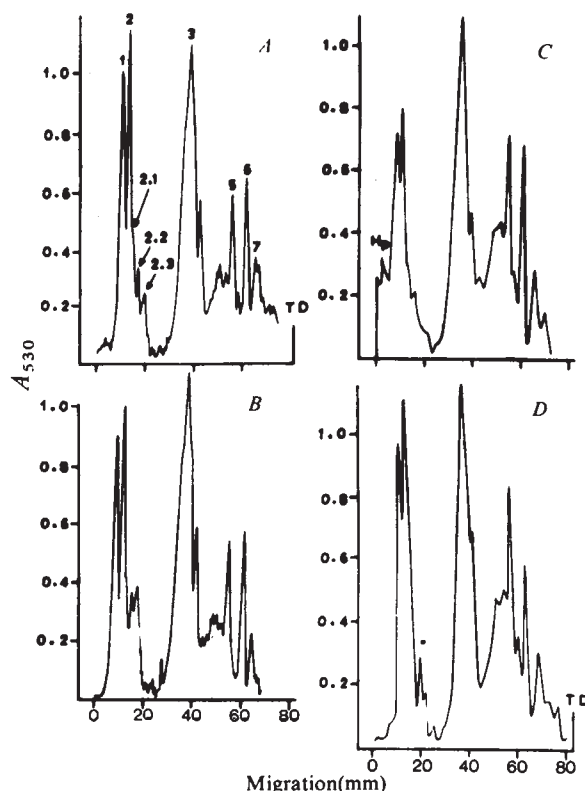


Fig. 1 Densitometer scans of gels stained for proteins with Coomassie blue after electrophoresis of red cell ghosts on SDS-polyacrylamide¹². A, Normal human erythrocyte ghosts; B, β -ME-treated normal erythrocyte ghosts; C, CML erythrocyte ghosts; D, β -ME-treated CML erythrocyte ghosts. The protein bands of the normal human erythrocytes are numbered according to the nomenclature of Fairbanks *et al.*¹². Similar gel patterns were obtained for erythrocytes of five healthy adults and six CML patients. TD, Tracking dye.

Table 1 Concentration of GSH in erythrocytes

Sample	n	GSH (mg per 100 ml packed cells)
Normal erythrocytes	8	62 $\pm$ 5
CML erythrocytes	6	34 $\pm$ 4

The concentration of GSH in erythrocytes was determined by the procedure of Beutler *et al.*²⁷. Values are expressed as mean $\pm$ s.d.; n, number of humans examined.

intramembrane particles¹⁸ and enhanced rate of transbilayer movement¹⁹ of phosphatidylcholine (PC).

Haest *et al.*⁷ observed that the cross-linking of spectrin achieved by treating human red cells with sulphhydryl oxidizing agents leads to loss of transmembrane phospholipid asymmetry. Therefore, we studied the transbilayer distributions of diacylglycerophospholipids: PC, phosphatidylethanolamine (PE) and phosphatidylserine (PS), in the membranes of both normal and CML human erythrocytes using phospholipase A_2 , from two different sources, as an external membrane probe^{20,21}. Table 2 shows that *Naja naja* phospholipase A_2 degraded only PC in the normal red cells, whereas in CML erythrocytes this enzyme also hydrolysed PE (~27%) and PS (~30%). The degradation of PE and PS was not due to penetration of the enzyme into CML erythrocytes, because the extent of hydrolysis of these phospholipids remained almost constant even after prolonged incubation (2 h) of these cells with the enzyme (Table 2).

The presence of PS in the outer leaflet of the CML erythrocyte membrane bilayer was confirmed by using pancreatic phospholipase A_2 to probe the membrane phospholipid organization in the cells. The data in Table 3 indicate that this enzyme did

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Table 2 Erythrocyte phospholipid degradation by phospholipase A₂ obtained from *N. naja* snake venom

Sample	Incubation time (h)	PC (%)	PE (%)	PS (%)
Normal erythrocytes	1	56.43 ± 3.59	0	0
	2	56.82 ± 3.34	0	0
CML erythrocytes	1	41.10 ± 2.37	26.66 ± 1.62	30.41 ± 3.31
	2	42.08 ± 2.74	27.02 ± 0.76	30.33 ± 1.05

Erythrocytes were incubated with *N. naja* phospholipase A₂ for 1 and 2 h in conditions described previously²⁸. The enzyme reaction was stopped by washing the cells three times with PBS containing 10 mM EDTA. The extent of haemolysis was determined at the end of each incubation before the EDTA wash as described by Roelofsens *et al.*²⁹. Less than 5% of the cells were lysed in the experimental conditions. Lipids from the washed cells were extracted by the method of Rose and Oklander³⁰ but without a haemolysis step. The lipid mixture was chromatographed on silica gel G-60 TLC plates as described previously²⁸ and the total phosphorus content in each spot was determined³¹. The percentage of the total phospholipid hydrolysed after treatment of red cells with the enzyme was determined by measuring the ratio of remaining diacylglycerophospholipids to the corresponding lyso derivative. Values shown are the mean of five determinations $\pm$ s.d.

Table 3 Erythrocyte phospholipid degradation by phospholipase A₂ obtained from hog pancreas

Sample	Incubation time (min)	PC (%)	PE (%)	PS (%)
Normal erythrocytes	45	2.91 ± 0.60	0	0
	90	3.57 ± 0.65	0	0
	135	4.71 ± 1.60	0	0
CML erythrocytes	45	30.72 ± 1.81	19.96 ± 1.57	28.76 ± 2.44
	90	38.07 ± 1.00	24.31 ± 1.36	28.45 ± 2.78
	135	39.77 ± 2.23	24.98 ± 1.04	27.94 ± 3.99

Treatments of erythrocytes with pancreatic phospholipase A₂ (Boehringer Mannheim) were carried out according to the method of Haest *et al.*⁷. The amount of enzyme used was 10 IU per 0.25 ml of packed cells. Incubations were for 45, 90 and 135 min at 37°C. The extent of haemolysis was <1% in the experimental conditions. The enzyme reactions were stopped, cells extracted and amount of degraded phospholipids determined, as described in Table 2. Values are the mean of four determinations $\pm$ s.d.

not attack the normal red cells, but readily hydrolysed PC (~40%), PE (~25%) and PS (~28%) in CML erythrocytes. This is quite consistent with the previous observation that pancreatic phospholipase A₂ hydrolyses only those red cells which contain PS on their outer surface^{7,22}.

As accessibility of different phospholipids to phospholipases in intact red cells has been correlated with their localization in the external leaflet of the membrane bilayer^{20,21}, we conclude that the typical asymmetric transbilayer phospholipid organization in the human erythrocyte membrane²³ is lost during CML. This conclusion is in accordance with earlier studies⁷⁻¹⁰ which showed that the abnormalities in erythrocyte spectrin are invariably associated with abnormal membrane phospholipid organization, and therefore support the view that the aminophospholipid-spectrin interactions are the major determinants of transmembrane lipid asymmetry in red cells⁷⁻¹⁰.

These observed abnormalities suggest that externalization of PS in CML erythrocytes could hyperactivate the blood coagulation system^{24,25}, and hence may cause thrombosis. Also, the defective erythrocyte cytoskeletal network would enhance

destruction of these cells by the spleen²⁶, making the patients severely anaemic.

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Transposable elements as mutator genes in evolution

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Strains of the bacterium *Escherichia coli* harbouring genes that increase mutation rates are known to have an evolutionary advantage in chemostat competition over otherwise isogenic strains with lower mutation rates¹⁻³. This advantage is frequency-dependent, the mutator strain being favoured only above a starting ratio of $\sim 5 \times 10^{-5}$, and it results from the fact that the necessary beneficial mutations cannot be generated in a mutator population below a certain size³. Here we consider the possibility that the mutagenic^{4,5} properties of transposable elements confer an advantage in the same manner as mutator genes. A previous report has shown that the transposon Tn5 increases the fitness of *E. coli* in chemostats, although the reason for this effect has not been established⁶. Our results show that the transposon Tn10 also confers an advantage in chemostats. In addition, we find that (1) this advantage, like that associated with mutator genes, is frequency-dependent, (2) whenever the Tn10 strains win, a segment of Tn10, probably its IS10 sequences, has undergone transposition to a new site, (3) the new insertions converge into a site contained within a 3.2 kilobase (kb) *PvuII* fragment of the genome, and (4) no transpositions are detected when the Tn10 population loses. We conclude that Tn10 confers an advantage by increasing the mutation rate of the host bacterium.

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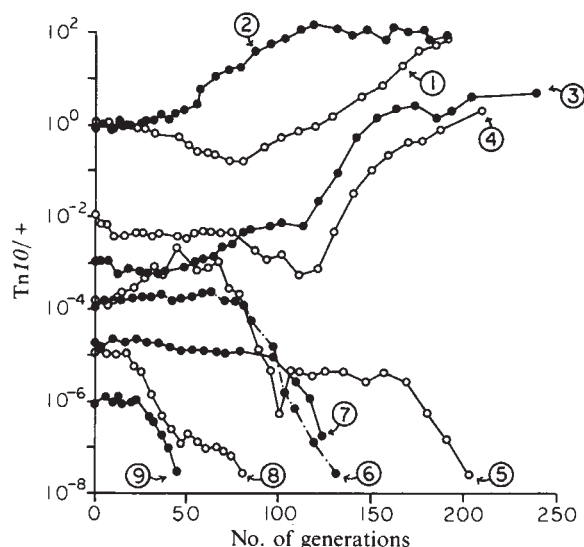


Fig. 1 Chemostats started with varying ratios of *Tn10* and wild type bacteria. Encircled numbers refer to chemostat numbers. Arrows denote times at which *Tn10* clones were retrieved from chemostats for further analysis. Holding volumes for the chemostats averaged 40 ml and generation times were 2.48 ± 0.13 h.

Tn10 is a 9.3-kb transposon consisting of two flanking 1.4-kb *IS10* insertion sequences, and a middle region with a 2.5-kb sequence coding for tetracycline resistance⁷. W3110*lac::Tn10*, the *Tn10* strain used in this study, is a W3110 strain made Lac by inserting⁸ *Tn10* into the lactose operon. An isogenic *Tn10* free W3110 strain, referred to here as wild type, was used as the competitor, and the two strains were distinguished experimentally by their Lac and tetracycline markers⁹. All experiments were performed in glucose-limited tetracycline-free chemostats³.

The population trajectories of nine chemostats started with different W3110 *lac::Tn10* to W3110 ratios are presented in Fig. 1. It is clear from these results that *Tn10* confers an advantage in chemostat competition. This outcome, however, like that observed in competition studies between wild type and high-mutating strains of *E. coli*³, is frequency-dependent, strongly suggesting that the advantage is through a mutagenic effect. Under this hypothesis, at starting ratios above 10^{-4} , the *Tn10* population produces more beneficial mutations and evolves faster than the wild-type population, while at lower ratios it is too small to produce the more fit mutant. The wild-type population 'wins' at the lower ratios because it continues to produce beneficial mutations by virtue of its larger size. We pursued this explanation further by analysing in more detail one *Tn10* clone isolated from each of the nine chemostats at the time points shown in Fig. 1. These clones were designated CH101-CH901, the first digit corresponding to the chemostat number.

To determine whether the entire *Tn10* sequence was undergoing transposition and thereby producing favourable mutations, we first checked for the presence of a new copy of the tetracycline determinant at other sites within the genome. This was done by crossing out the *lac::Tn10* marker from our nine *Tn10* isolates by P1 transduction, selecting the Lac⁺ transductants and determining whether the transductants were still tetracycline-resistant⁹. All of our isolates yielded tetracycline-sensitive Lac⁺ transductants. Thus, there is no second copy of the tetracycline resistance gene elsewhere in these genomes.

A mutagenic advantage, however, could still have been conferred by *Tn10* if it had induced deletions or inversions, or if the flanking *IS10* insertion sequences had undergone transposition. We tested these possibilities by Southern blot hybridization¹⁰. The results are presented in Fig. 2. Total DNA isolated from all nine clones was cut with *PvuII* (which does not cut within *Tn10*)⁷, resolved on agarose gels, transferred to nitrocellulose filters and probed with ³²P-labelled pRT61 (ref. 11), a

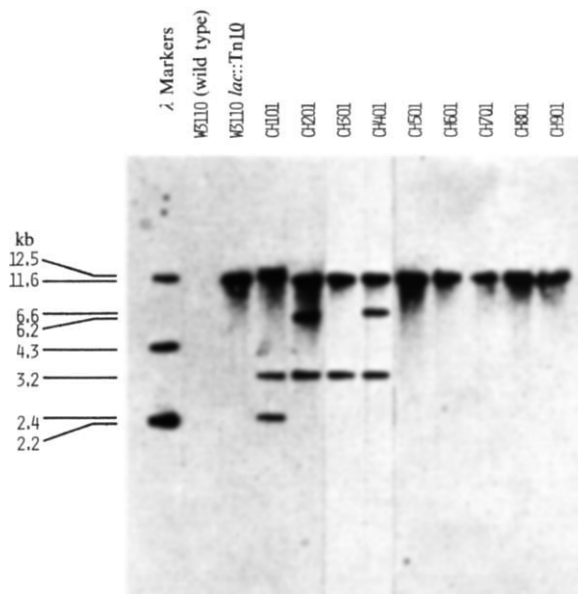


Fig. 2 Southern hybridization blots of total DNA from *Tn10* clones isolated from chemostats (see Fig. 1). All samples were cut with *PvuII* and probed with ³²P-labelled pRT61, a ColE1 plasmid derivative containing *Tn10*. Three λ markers are detected in the blot since pRT61 also carries some λ DNA. There is no homology between this probe and W3110, as lane 2 demonstrates. Clones CH101-CH401 were isolated from chemostats where the *Tn10* population won and clones CH501-CH901 from chemostats where it lost. The light band above the 12.5-kb band in the CH101 lane is due to incomplete digestion of the total DNA sample. For additional details, see text and Fig. 1 legend.

ColE1 plasmid carrying the entire *Tn10* sequence. Wild type *E. coli* does not normally carry copies of *Tn10* (ref. 12), thus we expected, and found, no bands in the lane containing W3110 DNA (Fig. 2). A clear pattern, however, emerged with the *Tn10* clones isolated from our chemostats. First, all nine clones, except CH101, still contained the original 11.6-kb band found in W3110 *lac::Tn10*. Chemostats 2-9 were started with the same clone denoted W3110 *lac::Tn10* in Fig. 2, whereas chemostat 1 was started with an independently isolated Lac⁻ strain carrying a *Tn10* insertion at a different site within the *lac* locus. This difference accounts for the presence of *Tn10* in a slightly larger (12.5-kb) fragment in clone CH101. Second, new bands were present, but only in *Tn10* clones isolated from chemostats 1-4, where the *Tn10* population had won. Third, although the new bands often varied in size, a 3.2-kb band was detected in every winning clone. Fourth, the fact that all the new bands were smaller than 9.3 kb, the length of *Tn10*, is consistent with our earlier transduction results suggesting that any new transpositions would have to be due to *IS10* sequences, not the entire *Tn10* transposon.

We conclude that *Tn10* confers an advantage by increasing the number of more fit mutants in the host population. The presence of the 11.6-kb or equivalent fragment in all nine isolates suggests that this advantage results from insertions (probably *IS10* insertions) into new sites, and not from deletions and inversions of host DNA generated by the original *Tn10* sequence. We cannot exclude the possibility that the convergence of some of the new insertions into a 3.2-kb *PvuII* fragment might be due to an insertion hotspot. However, given the frequency-dependent nature of our results and our observation that new insertions are detected only when the *Tn10* populations win, it is likely that the 3.2-kb fragment represents the site of a specific and beneficial new mutation. It is more difficult to determine the cause of the new insertions into the other *PvuII* fragments. As there is no evident pattern to their insertion sites, they could also be transpositions favoured by selection, or unselected transpositions triggered¹³ by the original 3.2-kb insertion.

Recently, it has been argued that transposable elements might be parasitic DNA evolved without marked selection for any phenotype at the level of the host organism and that transposition is mainly a mechanism for their dispersal within and between genomes^{14,15}. While this is an attractive hypothesis, the results presented here do not support it. Our results suggest that the mutagenic behaviour of the transposon *Tn10* is a strongly favoured phenotype, which, in the absence of any other selectable phenotype, is sufficient to account for the increase of the transposon in our bacterial populations. The possibility that transposable elements have an evolutionary role as mutator genes is appealing. As mutator genes normally can only be favoured through selection acting on a mutation located elsewhere in the genome, recombination in sexual populations works against their evolution by separating them from their mutations¹⁶. This effect led Leigh¹⁷ to postulate that if mutator genes have evolved in sexual populations, they are not likely to be general mutators, but ones with activity restricted to closely linked loci. The attractiveness of transposable elements is that they could have evolved as general mutators in sexual populations since recombination cannot easily separate them from the mutations they generate.

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Interaction between the *Xce* locus and imprinting of the paternal X chromosome in mouse yolk-sac endoderm

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In female eutherian mammals preferential inactivation of the paternally derived X chromosome (X^P) takes place in certain extra-embryonic tissues such as mouse yolk-sac endoderm, chorionic ectoderm and trophoblast^{1–3} and has been demonstrated both biochemically³ and cytologically^{1,2,4}. This is thought to be due to the paternal X chromosome being 'imprinted'^{5,9}, that is, somehow marked as different, during either male gametogenesis or fertilization, causing primary nonrandom X-inactivation in tissues that differentiate early, such as trophectoderm and primitive endoderm, from which yolk-sac endoderm is derived¹⁰. Different alleles of the X-chromosome controlling element, *Xce* locus, centrally located on the mouse

X chromosome, also cause primary nonrandom X-chromosome inactivation in embryonic tissues which would otherwise show random inactivation^{11,12}. The work reported here was designed to elucidate whether the nonrandom inactivation of the imprinted X^P in yolk-sac endoderm could be modified, or even overridden, by the effect of different *Xce* alleles. Using the modified Kanda^{13,14} method we have therefore studied the proportion of cells at metaphase with the X^P inactive in separated yolk-sac endoderm and mesoderm of mouse embryos heterozygous for a marker X chromosome (Cattanach's translocation) carrying different *Xce* alleles on X^P and X^M . The results show that the extreme *Xce*^c allele, when present on the paternally derived X, can significantly reduce the proportion of inactive X^P seen in yolk-sac endoderm compared with controls. This is the first evidence that imprinting of X^P is not an 'all or none' event but can be modified by a 'strong' allele at the *Xce* locus, and is another indication that the *Xce* locus may represent the inactivation centre.

Three alleles of the *Xce* locus are known, *Xce*^a, *Xce*^b and *Xce*^c, which affect the probability of X-chromosome inactivation in cells of the embryo proper in such a way that an X chromosome carrying an *Xce*^a allele is more likely to be inactivated than an X chromosome carrying an *Xce*^b allele in the same cell, and an X chromosome carrying an *Xce*^b allele is more likely to be inactivated than an X chromosome carrying an *Xce*^c allele in the same cell^{15–17}. It has been demonstrated both biochemically¹⁶ and cytologically¹⁷ that *Xce* effects are due to primary nonrandom inactivation and not to cell selection for or against a cell with a particular *Xce* allele active. To investigate *Xce* action in the extra-embryonic membranes, that is, possible interactions between imprinting of X^P and alleles at the *Xce* locus, female embryos heterozygous for Cattanach's translocation (X^T) were produced from crosses of different *Xce* genotypes. The two extreme alleles *Xce*^a and *Xce*^c were used. The *Xce* genotype of the parental mice had been verified by extensive progeny testing.

Embryos were recovered at 13½ days post-coitum, their yolk-sacs removed and placed in medium 199 with colchicine (4 µg ml⁻¹ final concentration) for 2 h to accumulate cells at metaphase, then separated into their component endoderm and mesoderm layers by partial digestion for 3 h in a mixture of 2.5% pancreatin and 0.5% trypsin (w/v) made up in Ca²⁺- and Mg²⁺-free Tyrode's solution at 4 °C followed by dissection with watchmaker's forceps. The separated cell layers were then individually treated by the modified Kanda method, as described elsewhere¹¹, and metaphase spreads produced.

All slides were coded and scored blind. Individual cells at metaphase from tissue from female embryos were scored for whether the dark staining inactive chromosome was the marker X (X^T) or the normal X chromosome (X^N) (Fig. 1a,b). The results are given in Table 1. For each cross, embryos from several different litters were used and the proportion of cells with X^P inactive differed significantly from litter to litter, when tested for heterogeneity of binomial proportions. This variability may be due to existing heterogeneity within the populations of mice used, because, due to breeding difficulties, neither the translocated X nor the PGK X chromosome carrying the *Xce*^c allele is maintained on an inbred background. To overcome this, a special analysis of variance method¹⁸ was used to determine differences between the crosses. There is no statistically significant difference between the two control reciprocal crosses, crosses 2 and 3, in which both X^P and X^M carry the *Xce*^a allele. These results confirm that in yolk-sac endoderm ~90% of cells have X^P inactive whereas there is more or less random inactivation in yolk-sac mesoderm. However, in cross 1, where the imprinted X^P carries the 'strong' *Xce*^c allele and X^M the *Xce*^a allele, the proportion of cells with X^P inactive has been reduced to 78% from control levels of around 90%. This represents a highly statistically significant difference from all three other crosses (see Table 1, $\chi^2 = 23.72$ with 3 degrees of freedom, $P < 0.0001$). In the reciprocal cross, cross 4, however, where X^M carries the *Xce*^c allele and X^P the *Xce*^a allele, the

Table 1 Results of crosses of different *Xce* genotypes

Cross	Genotype of female embryo X^M/X^P	No. of litters	No. of heterozygous female embryos	Endoderm			Mesoderm		
				X^P (%)	X^M	No	X^P	X^M	No
1	$X^T Xce^a/X^N Xce^c$	7	24	405 (78.0%)	114	61	258 (52.7%)	231	29
2	$X^N Xce^a/X^T Xce^a$	5	12	180 (92.8%)	14	16	126 (55.5%)	101	19
3	$X^R Xce^a/X^N Xce^a$	8	25	415 (89.8%)	47	61	220 (52.0%)	203	31
4	$X^N Xce^c/X^T Xce^a$	3	12	127 (92.7%)	10	20	115 (67.2%)	56	12

The values represent the number of cells in heterozygous female embryos from each cross with either X^P or X^M dark-staining and inactive in metaphase cells from separated yolk-sac endoderm and mesoderm. The first column is the number of cells with X^P inactive, the second column the number of cells with X^M inactive and the third column the number of cells in which no dark-staining X chromosome was seen. The number in parentheses below X^P is the proportion of cells with X^P inactive calculated as a percentage of the total number of cells that did show a dark-staining X-chromosome. Crosses 2 and 3 are the two control crosses in which both X^M and X^P carry the *Xce*^a allele, but in cross 2 the translocated X is inherited from the father and in cross 3 it is inherited from the mother. Crosses 1 and 4 are the reciprocal experimental crosses; in cross 1 X^M carries *Xce*^a and is the translocated X chromosome and X^P carries *Xce*^c; in cross 4, X^M carries *Xce*^c and X^P carries *Xce*^a and is the translocated X chromosome.

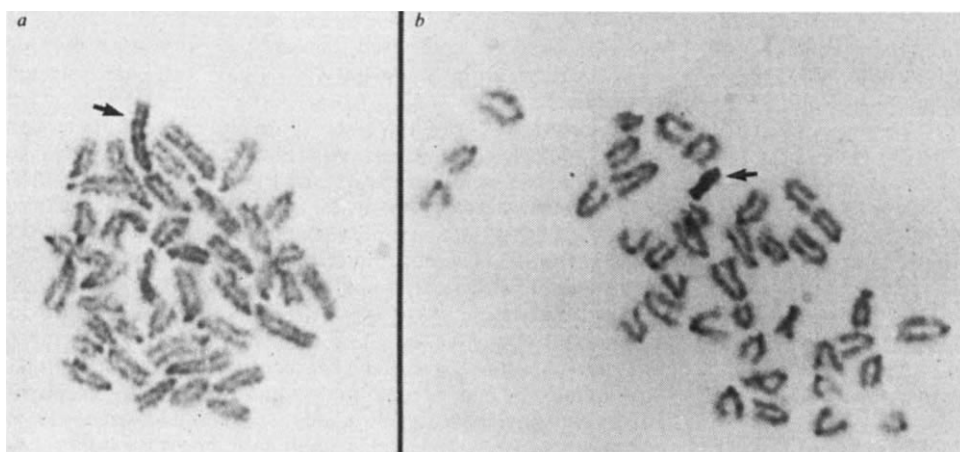


Fig. 1 *a*, A metaphase spread from the extra-embryonic endoderm of a female embryo heterozygous for Cattanach's translocation treated by the Kanda method in which the dark-staining inactive X is the translocated chromosome. *b*, A metaphase spread from the same embryo in which the dark staining X is the normal X chromosome.

proportion of endoderm cells with X^P inactive is not significantly different from control (homozygous *Xce*^a) levels.

The expected *Xce* effects in the mesoderm cells where imprinting is not a factor are less clear. Classical *Xce* effects as measured in the whole embryo^{16,17} would predict an increase in the proportion of cells with X^P inactive compared with controls in cross 4 and a decrease in the proportion of cells with X^P inactive in cross 1. Our results show a trend in the expected direction in the mesoderm of cross 4, which is nevertheless not statistically significant, and no trend in the opposite direction in cross 1. This lack of coincidence with classical whole-embryo *Xce* expectations may be due to tissue-specific factors operating in yolk-sac mesoderm and in no way invalidates the modifying effect of the *Xce* locus on the nonrandom inactivity of X^P previously thought to be the norm in yolk-sac endoderm.

These results show that alleles of the *Xce* locus can interact with imprinting in such a way that the extreme *Xce*^c allele when inherited on the X^P in an $X^P Xce^c/X^M Xce^a$ heterozygote can reduce the effects of imprinting, as expressed by the proportion of cells in the yolk-sac endoderm that have X^P inactive; when the *Xce*^a allele is carried on the X^P , however, in an $X^P Xce^a/X^M Xce^c$ heterozygote, the effect of imprinting cannot be increased from that seen in control levels in *Xce*^a/*Xce*^a homozygotes. This could be interpreted to mean that in the control *Xce*^a homozygotes the X^P is fully imprinted; the paternal X can thus be made less than fully imprinted but not more than fully imprinted.

This work, conservatively interpreted, shows that alleles at the *Xce* locus can interact with imprinting of the paternal X chromosome to determine the extent of nonrandom inactivation

of X^P . At the time of X-inactivation we envisage that controlling centres on each of the two X chromosomes in the female embryo compete for a factor that either initiates X-inactivation or guarantees continued activity of the X. Imprinting of the paternal X chromosome thus is seen as modifying the controlling centre on the X^P so as to make it more likely to become inactivated. We suggest that the *Xce* locus itself is the major controlling centre on the X chromosome which is modified by the effects of imprinting; an *Xce*^c allele thus represents a controlling centre which is more resistant to the effects of imprinting than is an *Xce*^a allele. This suggestion could be evaluated by investigating whether in the extra-embryonic membranes of *Xce*^c homozygotes one finds preferential inactivation of X^P (as seen in *Xce*^a homozygotes) or whether the presence of the *Xce*^c allele *per se* at the time of imprinting is sufficient to disturb preferential inactivation of X^P . However, very close linkage between the *Xce* locus and the translocation breakpoint¹⁵ has so far prevented the production of animals carrying a $X^T Xce^c$ chromosome.

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Failure of filipin to detect cholesterol-rich domains in smooth muscle plasma membrane

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Using the cholesterol probe filipin and freeze-fracture electron microscopy, Montesano¹ reported an apparent difference in cholesterol content between contiguous zones of the smooth muscle plasma membrane. In this membrane, microinvaginations termed caveolae are organized in band-like domains^{2–6}, which were highly sensitive to filipin (and therefore cholesterol-rich) compared with the surrounding non-caveolar inter-band regions (assumed to be cholesterol-poor)¹. Using another cholesterol probe, the saponin tomatin⁷, applied alone and after filipin treatment, we now present evidence that both the band and inter-band domains of the smooth muscle plasma membrane are cholesterol-rich. This leads to the conclusion that the lack of inter-band response to filipin is a false-negative cytochemical result.

Our studies were carried out on the smooth muscle of rabbit aorta as described in Fig. 1 legend. Caveolae in this and other tissues^{2–6} are seen as flask-shaped in-pocketings of the plasma membrane (Fig. 1a) aggregated into band-like clusters on its surface (Fig. 1b). After treatment with filipin, distinct circular deformations (~25 nm diameter), indicating the presence of multimolecular filipin-cholesterol complexes⁸, are formed in

the membrane (Fig. 1c). As in rat intestinal smooth muscle¹, the caveolar bands of rabbit aortic smooth muscle reveal numerous filipin-induced deformations whereas the inter-band regions are comparatively resistant to the agent. By contrast, however, treatment with tomatin causes extensive perturbation of the inter-band membrane and affects only limited peripheral areas of the bands (Fig. 1d). Interaction of tomatin with cholesterol produces long hemitubular deformations⁷ whose width (40–60 nm) often exceeds the space available between adjacent caveolae. The close-packing of the caveolae seems to

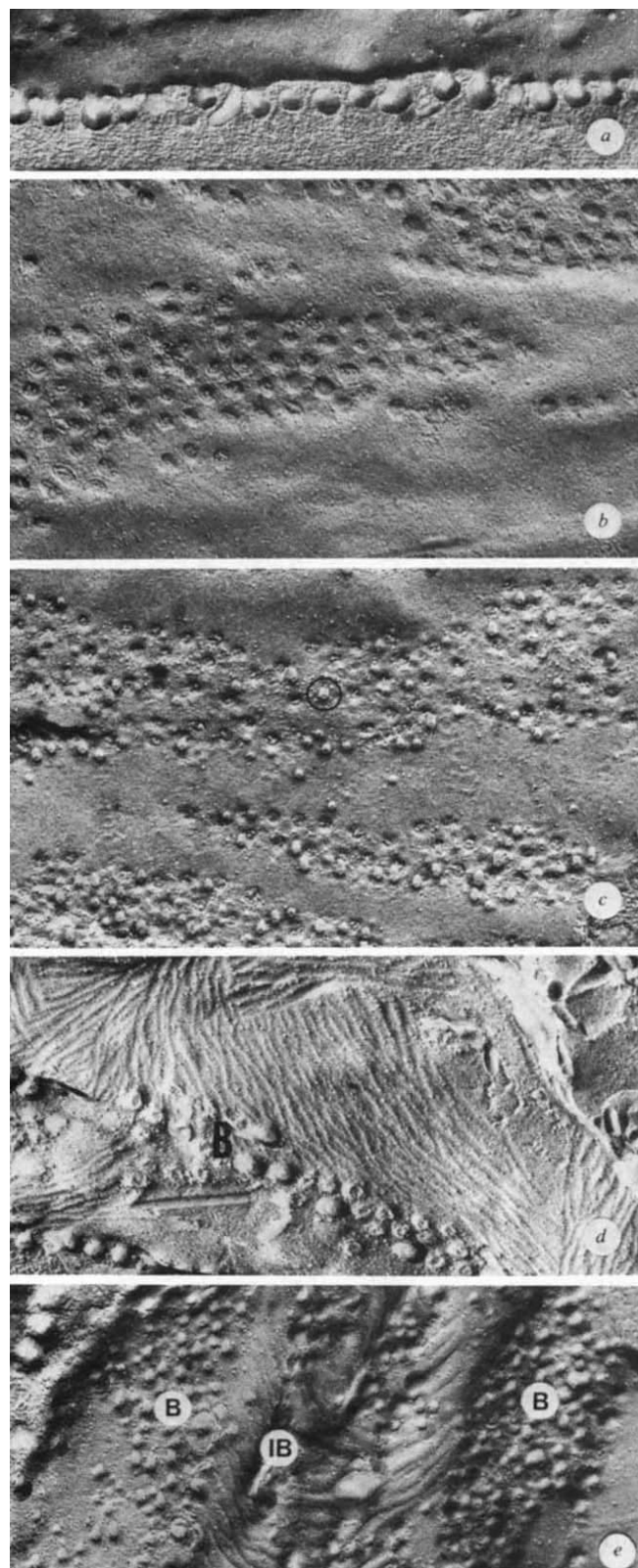


Fig. 1 Freeze-fracture replicas of control and experimentally treated aortic smooth muscle plasma membranes. *a*, Control, showing flask-shaped structure of caveolae in side view. *b*, Planar view (P-face)¹³ of the membrane in a control. Caveolae are seen as circular depressions (representing their cross-fractured necks) aggregated into distinct bands which are separated by smooth inter-band zones. *c*, Filipin-treated specimen (P-face). Filipin deformations (example encircled) are induced predominantly in the bands and can be distinguished from caveolae by their upward projection and smaller size. *d*, Tomatin-treated specimen (E-face) showing extensive corrugation of the inter-band membrane by this agent. Caveolar bands (B) are resistant. *e*, Specimen treated sequentially in filipin and tomatin (P-face view). Filipin deformations occur mainly in the bands (B), and tomatin deformations predominantly in the interbands (IB), so that alternate zones affected by each agent are seen. $\times 38,400$.

Methods: Aortas were rapidly removed from anaesthetized or freshly killed New Zealand White rabbits and placed immediately in cacodylate-buffered 2.5% glutaraldehyde. Small tissue samples were taken from the ascending and descending segments of the vessel wall and fixed in glutaraldehyde for 1 h before incubation in fresh fixative solutions containing cholesterol-binding agents¹⁴. Three experimental treatments were given: (1) filipin alone, (2) tomatin alone and (3) filipin followed by tomatin. Filipin (Upjohn) was used at a concentration of $100 \mu\text{g cm}^{-3}$, and tomatin (Serva) at $150 \mu\text{g cm}^{-3}$. The experimental solutions contained 1% dimethyl sulphoxide (DMSO). Incubations were continued for 3, 5 or 22 h in light-proof vials at 20 °C. Controls received treatment in fixative containing 1% DMSO. All specimens were cryoprotected in cacodylate-buffered 25% glycerol, frozen by immersion in propane (-180°C), and freeze-fractured close to the tissue surface using a double replica device in a Balzers BAF 400T unit.

Table 1 Quantitative analysis of the response of the smooth muscle plasma membrane to filipin and tomatin

Band regions	Filipin (single treatment)	—NS—	Filipin/tomatina (double treatment)		—S—	Tomatin (single treatment)	
	91 ± 6(30)		96 ± 10/3 ± 1(12)			28 ± 4(30)	
	S		S S			S	
Inter-bands	37 ± 4(30)	—NS—	25 ± 6/40 ± 4(12)		—S—	66 ± 3(30)	
Total plasma membrane	62 ± 4(30)	—NS—	64 ± 16/26 ± 4(12)		—S—	58 ± 4(30)	

Comparison of the response to each agent after double treatment (central column) with that observed after the corresponding single treatments (filipin, left column; tomatin, right column) in: (1) the caveolar band regions, (2) the inter-band zones and (3) the total plasma membrane. The response to filipin was assessed by determining the numerical density of filipin deformations per μm^2 , and that to tomatin by measuring the percentage area of membrane corrugated by the agent. (The tomatin effect cannot be expressed as a numerical density because of the irregular size of the deformations induced by this agent.) Values are the mean $\pm$ s.e.m. Statistical analysis was carried out using Student's *t*-test. S indicates significant differences ($P < 0.001$); NS, not significant. Numbers in parentheses show numbers of cells analysed (taken from six animals for each experimental treatment). Note that there is no significant difference between the effect of filipin in samples treated with filipin alone and those treated with filipin followed by tomatin. The response to tomatin, however, does show a reduction in samples receiving the double treatment compared with those exposed only to the saponin. This reduction is marked in the bands but slight in the inter-bands, reflecting the relative quantities of cholesterol in each region bound by the previous exposure to filipin. The data shown come from a representative series of experiments in which exposure to tomatin was for 5 h, and that to filipin was for 22 h. These periods were optimal; longer tomatin treatments produced disruption of some membranes and shorter treatments with filipin left deeper cell layers unpenetrated by the agent. Analysis of cells remaining undisturbed following 22 h treatment with tomatin, and of those cells affected after 3 or 5 h exposure to filipin, gave a similar response pattern.

act as a physical constraint on deformation of the bands by tomatin.

The effect observed with tomatin indicates that either the inter-bands are cholesterol-rich or that lateral translocation of cholesterol from band to inter-band occurs during exposure to tomatin. To distinguish between these possibilities we carried out double-labelling experiments, treating first with filipin to bind the cholesterol present in the bands, and then with tomatin to discover whether the inter-bands remained sensitive to the saponin. This they did, as can be seen from Fig. 1e and Table 1, giving a response similar to that obtained with tomatin alone.

As both filipin and tomatin interact specifically with 3- β -hydroxysterols^{7,9}, the predominant species of which is cholesterol in mammalian cellular membranes¹⁰, our results cannot be explained by differences in specificity of action between the two agents. It also seems unlikely that differences in sensitivity are involved, as filipin is slightly more sensitive to low cholesterol concentrations than is tomatin⁷. Our findings therefore strongly suggest, contrary to prevailing opinion¹, that the distribution of cholesterol in the smooth muscle cell plasma membrane is in fact homogeneous, at least at the level of resolution afforded by freeze-fracture cytochemistry. As the resistance of the inter-band membrane to filipin cannot be attributed to a dearth of cholesterol, it must be considered, from the cytochemical point of view, a false-negative result. The factors responsible for this resistance remain uncertain; closely-packed intramembrane particles—which may have an inhibitory effect on filipin action¹¹—are evidently not involved because the inter-band regions are sparsely covered in intramembrane particles compared with the bands³⁻⁶. However, recent evidence suggests that dense membrane-associated (that is, peripheral) protein components may also influence the effects of filipin¹², and a prominent structural feature of the smooth muscle plasma membrane is the presence of dense myofilament-insertion plaques attached to the cytoplasmic surface of the inter-bands^{2,6}. If these plaques form a mechanical scaffold, rendering the membrane too rigid to be easily deformed, then the occurrence of caveolae in discrete zones and the preferential formation of filipin deformations in these zones could have a common explanation.

Filipin is now widely used as a sterol probe in freeze-fracture cytochemistry on the assumption that the deformations induced faithfully reflect the planar distribution of membrane sterols. Our present findings demonstrate that this assumption is not always valid. Negative filipin results may, however, be verifiable using saponins.

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Corrigendum

Correction to 'Geomagnetic secular variation as a precursor of climatic change' by V. Courtillot, J. L. Le Mouél, J. Ducruix & A. Cazenave: It has recently been brought to our attention that there was an error of sign on the ordinate axis of Fig. 1 of our paper¹. This should read $d(\Delta T)/dt$ and not $d(\Delta T)/dt$. This error was made only in the figure and the discussion in the text is based on the correct sign. Thus our conclusions remain unaltered.

The error unfortunately also occurred in two earlier papers^{2,3}. The original data are found in Morrison's⁴ Fig. 4. $d(\Delta T)/dt$, termed the excess length of day, is actually measured in ms per SI day. It may be clearer, as stated in the legend to Fig. 1 of the paper by Le Mouél *et al.*² to express $-d(\Delta T)/dt$ as the fractional increase in the Earth's rotation rate $\Delta\Omega/\Omega = m$. This requires multiplication by 1.157×10^{-8} . Thus, for instance, m decreases from about 0 to about -3.5×10^{-8} between 1930 and 1970.

We take the opportunity of this correction to mention work in progress on the correlation between the fractional increase in the Earth's rotation rate and the westward drift of the geomagnetic field. This suggests that the rotation lags the field by 15 rather than 10 years. Thus, the forecasted acceleration in rotation following the 1970 secular acceleration jerk might not occur before 1985 and the increase in average global temperature before 1995–2000.

We thank H. A. Taylor Jr, H. E. Mayr and L. Kramer for pointing out the need for this correction, and M. G. Rochester for comments on the subject.

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Most dangerous reaction of all

Frank Barnaby

The Soviet Union and the Arms Race. By David Holloway.
Yale University Press: 1983. Pp.210. £7.95, \$14.95.

WHY is the nuclear arms race allowed to go on, when it is increasing the probability of a nuclear world war? There are two possible reasons. The political leaders of the super-powers may actually want a nuclear-war-winning (i.e. first-strike) capability to further their ambitions for world domination or strategic superiority. Or the nuclear arms race may simply be out of political control.

We know a great deal about American nuclear weapons and nuclear thinking from official and semi-official American sources. To me the evidence seems to suggest that in the United States the nuclear arms race is out of political control. The military-industrial-academic-bureaucratic complex has grown too strong for the American President to resist. But what about the Soviet Union? Could this be true there too, or does the Soviet leadership hanker after a first-strike capability? The USSR is so obsessively secret about all military matters that we can say next to nothing from official information.

Unfortunately, in *The Soviet Union and the Arms Race* David Holloway doesn't answer this most vital question. At least not directly. There are excellent descriptions of Soviet military technological developments and Soviet thinking about nuclear weapons and nuclear war, but we do not learn what really drives the Soviets to add further weapons to nuclear arsenals that are already irrationally large and to develop and deploy increasingly destabilizing nuclear weapons and supporting technologies. We are told that the Soviets have reacted to American nuclear-weapon deployments, and that the action-reaction phenomenon plays some part in Soviet nuclear decision-making. And the evidence presented for this view is convincing. The history of the early development of nuclear weapons in the USSR is particularly well described. We are also told that the USSR developed strategic nuclear power as the main symbol of Soviet super-power status. The USSR is regarded as America's super-power equal because of Soviet ballistic missiles, even though its gross national product is only a half that of the United States. This is obviously not the whole story, however, because the purpose would be served with a smaller nuclear arsenal.

The Soviet Union and the Arms Race shows very clearly that the Soviet leaders see nuclear weapons as "both instruments of power and influence" but that they realize the awesome consequences of nuclear war and are anxious to avoid it. It seems, though, that the leadership is as

impotent as Western leaders to slow down and reverse the arms race. We are told that arms control talks are not the answer unless they are accompanied by unilateral steps to slow down the arms race. Quite so. But one must conclude that the Soviet leaders are as unable to take unilateral steps as Western leaders.

According to Holloway, the Reagan approach — of refusing to admit equal status to the Soviet Union and branding it

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REASONS

Cruise to nuclear victory? A YBGM-109 Cruise missile rises out of the Pacific after an underwater launch off the Californian coast.

an "evil empire" — exaggerates both the external strength and the internal problems of that country. The USSR, he says, is at a turning point in its history. Current economic problems will force the leaders to make difficult yet crucial decisions about the allocation of resources, the system of economic planning and management, and so on. It is just at such a time when the West should be prepared for cooperation rather than confrontation; a time when better East-West relations may be evolved and pursued. Given the heavy burden the Soviet military effort is placing on the economy, Soviet leaders may prefer to free some of the resources to contribute to economic growth and improve the living standards of the workers. There is,

however, little reason to doubt that the Soviet leaders will respond to any challenge to a new round in the arms race by increasing their military power. They are not going to be humiliated again as they were in the 1962 Cuban missile crisis.

Reading *The Soviet Union and the Arms Race* leaves one with the distinct impression that the nuclear arms race may be out of the control of Soviet leaders. Beyond this conclusion, it is clear that the scientific profession should look into the consequences of advances in military science. Recently, a group of eminent Soviet Academicians suggested that the most destabilizing military technologies, such as the use of high-energy lasers in space in anti-ballistic missile systems, should be discussed by an international group of scientists. Scientists should indeed have a special interest in discussing the arms race. At least 500,000 research scientists are, after all, working in military research and development. The activities of this group, representing about 25 per cent of the world's research scientists, are mainly responsible for the Soviet-American nuclear arms race. If there were no military science, this arms race would, at least qualitatively, soon grind to a halt.

There is a growing consensus that the nuclear arms race is the main threat to world security. That the deployment of more accurate and reliable nuclear weapons, with greater targeting flexibility, is causing the United States to change its nuclear policy from one of nuclear deterrence by mutual assured destruction to nuclear-war fighting is common knowledge. With the deployment of Trident-II submarine-launched ballistic missiles, five or so years from now, this change will be complete. Already, extremists are arguing that a nuclear victory is possible, and that nuclear strategies should be modified accordingly. Great efforts are being made to develop effective anti-submarine warfare, anti-ballistic missile and anti-satellite warfare techniques. So great are these efforts that it is hard to believe that they will not succeed. If they do, or are perceived to, then the views of those who believe a nuclear war can be won will almost certainly come to dominate. Many believe that the progression from nuclear deterrence by mutual assured destruction to nuclear-war fighting to nuclear-war winning is considerably increasing the probability of a nuclear world war.

The Soviet Union and the Arms Race, a most valuable contribution to the nuclear debate, shows that Soviet policy is likely to follow the American path. This move in the American action-Soviet reaction pattern may prove to be the most dangerous of all.

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Little and large

Carole Jordan

Atoms in Astrophysics.

Edited by P.G. Burke *et al.*

Plenum: 1983. Pp.350. \$49.50, £34.65.

THE interaction between atomic physics and astrophysics has its origins in work of the nineteenth-century spectroscopists, who used stellar spectra to establish empirical formulae to describe the series of lines observed. Thus began a fruitful dialogue — discoveries from astrophysical spectra stimulating atomic physics, atomic theory providing the means by which quantitative analyses and further astrophysical progress could be made. Since its foundation by H.S.W. Massey, this cross-fertilization has been a recurring theme in the work of the atomic physics group at University College London. The group is now under the leadership of Professor Michael Seaton, whose sixtieth birthday is celebrated by the publication of this book.

The review articles in *Atoms in Astrophysics* are all by Seaton's colleagues, collaborators and past students. Naturally they emphasize those areas where Seaton has made his most significant contributions but in doing so they cover a wide range of topics. The early chapters on scattering theory by Burke and Eissner, and by Norcross, and the article on long-range potentials by Peach will interest

specialists in atomic theory. The later essays, which concentrate on applications to planetary nebulae and hot plasmas, will be of particular value to astrophysicists.

One cannot think of quantum defect theory, ably reviewed by Moores and Saraph, without being aware of Seaton's contributions over many years. The great potential of quantum defect theory was apparent even in the early work and it still finds an application in modern calculations associated with autoionization and dielectronic recombination, both of importance to astrophysicists. Dubau and Van Regemorter review applications of atomic theory to the interpretation of the spectra of hot plasmas in astrophysical sources and laboratory nuclear fusion devices. Since the early 1960s observations from space in the extreme-ultra-violet and X-ray spectral regions have led to an increasing requirement for a wide range of atomic data. The progress of the atomic theory has in several instances been stimulated by discoveries made first from solar spectra. This close liaison between solar physics and atomic physics is now bearing fruit also in the context of the interpretation of EUV spectra of a wide range of astrophysical objects, obtained with the International Ultraviolet Explorer satellite. Seaton's own astrophysical interest has been mainly in planetary nebulae, discussed here by Flower. Seaton applied many of his early calculations of collisional excitation cross-sections to forbidden transitions in these low density nebulae.

It has always been Seaton's hope that simple methods of computing atomic data could be developed to encourage astronomers to make their own calculations of rates required. The UCL program SUPERSTRUCTURE is discussed by Nussbaumer and Storey. It is now used at several locations in Europe and the United States, and has gone some way to satisfy that aim. However, other approaches to the same concept continue to be required and the more general state of such programs and of data banks is discussed by Burke and Eissner.

The volume would not have been complete without the account by Bates of the historical development of research on the forbidden atomic lines in auroral spectra. It was in this context 30 years ago that Seaton applied his early calculation of excitation by electron impact. A sentence by Bates, if generalized beyond auroral spectra, expresses the importance of atomic physics to astrophysics — "The results that Seaton provided initiated the slow advance . . . from the promiscuity of qualitative reasoning to the discrimination of quantitative reasoning". *Atoms in Astrophysics* sets out in a historical context some of the methods by which this has been achieved. [1]

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At the frontiers of simple systems

William Klemperer

Chemical Dynamics via Molecular Beam and Laser Techniques.

By R.B. Bernstein.

Oxford University Press: 1982. Pp. 262.

Hbk £25, \$49.50; pbk £10.95, \$19.50.

R.B. Bernstein has brought many new methods and insights to the study of the dynamics of chemical reactions. This monograph is a most enjoyable transcription of his 1980 Hinshelwood lectures and conveys well their excitement. The lectures are a well balanced blend of experimental and theoretical methods used to probe, in essence, the non-statistical features that may occur in chemical reactions. This, then, is not a textbook of chemical dynamics; rather it is a tour guide to the frontiers of the subject.

Bernstein describes the methods and results of quantum state preparation of reactants and also quantum state analysis of products. A great deal of discussion is devoted to the most important question of vibrational energy selectivity in the rates of chemical reactions. The account of reactions whose dynamics can be treated by classical motion on a single potential surface follows a chapter on non-reactive scattering. The question of inversion, namely obtaining the potential from observation is treated in both of these chapters. There is, of course, also a chapter on the information theoretical approach, which provides the balance to detailed trajectories on a potential surface. All of the nine chapters are lucidly written and include up-to-date reference lists of relevant review articles.

At this stage in the development of chemical dynamics, the emphasis in the subject is on simple systems. Most of the systems studied involve diatomic species, where there is a single rotational and a single vibrational quantum number. Studies examining the selectivity of reaction rates towards vibrational, rotational and translational energy can be systematically pursued, as Bernstein illustrates by using a number of well-chosen examples.

The lectures deal with low-pressure gas-phase reactions of extreme simplicity, and the reader of this monograph will certainly gain knowledge about what is feasible in the investigation of the details of simple chemical reactions. However the book also provides insights which will be valuable in guiding the study of more complex systems. In this sense it should appeal to a wide audience. [1]

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Journals' review issue 1983

On October 6th *Nature* will publish the third review supplement devoted to science journals. The two previous supplements (covering journals first published between January 1978 and May 1980, and June 1980 and May 1981) appeared in *Nature* 293, 341–369 (1981) and 299, 491–514 (1982).

Criteria for inclusion of a journal in the 1983 issue are that:

- the first number appeared, or the journal was re-titled, between June 1981 and May 1982;
- it is published at least three times a year;
- the main language used is English.

Broadly, periodicals of professional interest to scientists will be considered for review, with the exception of abstracts' journals. Journals appearing prior to June 1981 but after January 1978, and not covered in the previous issues, will also be considered.

Publishers and societies are invited to submit four sample issues of journals satisfying the above criteria, including the first and most recent numbers, to the Review Editor, *Nature*, 4 Little Essex St, London WC2R 3LF, England (London 836 6633 ext 2570) as soon as possible.

Doing what comes naturally

Richard Mabey

Man and the Natural World: Changing Attitudes in England 1500–1800.

By Keith Thomas.

Allen Lane/Pantheon: 1983. Pp.425.

£14.95, \$19.95.

IN THE preface to this expanded version of his 1979 Trevelyan Lectures, Keith Thomas thanks his audience for enduring "conditions so wintry as to remind all present that man's conquest of the natural world is still far from complete". Old-fashioned awe is an uncommon commodity these days, and Thomas does not find much evidence of it in this survey of three centuries of our relationships with the natural world. Subjugation may have given way to stewardship, but man has remained firmly in the saddle.

Thomas's argument, exhaustively documented (there are, at a rough count 4,000 references) and elegantly composed, is nonetheless very simple. In the "early modern period" a series of contiguous intellectual revolutions began to undermine our belief that the rest of the natural world had been created solely for our own use and spiritual enlightenment. The cracks in this Christian anthropocentrism widened with the birth of an apparently more objective natural philosophy in the eighteenth century (pioneered ironically by a new breed of rationalist clergy), which viewed the natural world as having an existence in its own right, and led inevitably to the love affairs with nature of nineteenth-century Romantics and twentieth-century conservationists.

At the time these new perceptions were deeply unsettling. They weakened the boundary which separated us from the rest of creation, and which was used to define and justify our actions. And their after-shocks help to explain the perplexity we still feel in trying to evolve a code of ecological ethics for a species which is simultaneously natural and self-consciously cultural. But some shifts in sensibility were undoubtedly permanent. We are now sufficiently acquainted with the beastly nature of our

own bodies, for instance, to be able to laugh at earlier struggles to reconcile the idea of Divine Creation with the realities of defaecation. Thomas quotes one Stuart theologian: "... Yet I will be a more noble creature; and at the very time when my natural necessities debase me to the condition of the beast, my spirit shall (I say at that very time!) rise and soar ...".

But I think Thomas misjudges how much this reflection represented widespread changes in social attitudes and how much the shifting moods of a particular, educated class. Although responsibility by privilege replaced subjugation by right, we went on behaving in much the same way. Even aboard spaceship Earth, some animals are more equal than others. How, for instance, do we categorize the scientific culling of species, justified as being done for the animals' (or the ecosystem's) "own good"? And though Thomas makes up an horrific catalogue of early cruelties, we have to face the facts of our own more subtle tortures. In country sporting enclaves, heads are still bitten off live animals, and cock-fighting and badger-baiting flourish. And out of sight in another sense we have added a whole new set of ordeals, from the factory farm to the aerial crop-spray, that might well turn the stomach of a country-born, seventeenth-century burner of cats.

These examples could be rightly challenged as anecdotal, and not the way to write history. Yet it must be said that Thomas's card-index approach to history, culling tales and evidence from diaries, cookery books, poems, is also, precisely, anecdotal; and that to group this evidence in accordance with its superficial resemblances (much as Victorian anthropologists used to "collect" and group types of human behaviour, regardless of context) produces a version of history where change appears to be arbitrary and subjective. On the matter of trees, for example, Thomas's argument requires a gradual increase in respect for trees and woodland. His carefully selected evidence (laments over felling, eulogies to the "spirits" of trees) obscures the statistical fact that the eighteenth century was a period of intense woodland clearance, only rivalled by the past 40 years. Again, the practice of lopping trees for firewood was not discontinued in the eighteenth century

because, as Thomas suggests, it was seen as a "violation" of trees (it actually extended their lives) but because economic and political measures had led to the extinction of commoners' rights to wood in favour of the landowners' rights to timber.

The neglect of the idea that attitudes are determined as much by facts of ownership, working relationships and physical intimacy as by period or fashion is the major weakness of this book. It is especially sad that we are given so little evidence about those whose relationships with nature were most intimate — the landworkers — for it is in their pre-rational view of the world, with its fears and folklore, its ecological magic and sense of natural affinities, that the green revival has its roots as much as in the "modern" understanding documented by Thomas. But they are eclectic roots, and perhaps this is the spirit in which to approach what is, for all its rather glib generalizations, a compulsive and fascinating book. It is a kind of bestiary of our historical views of nature, an anthology of our persistent, obsessive, inquisitive tinkering with the Earth, a salutary reminder that we couldn't stop behaving like this without ceasing to be human, so we had better do it well. □

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Beyond conception?

W.D. Billington

Immunological Fertility Regulation.

By Warren R. Jones.

Blackwell Scientific/Blackwell Mosby:

1983. Pp.270. £29.50, \$41.95.

ECONOMIC and social factors continue to dictate the pressing need for a reduction in human population increase at the global level. Despite this, it is an uncomfortable fact that there is no totally acceptable and safe contraceptive procedure currently available for use either in Western societies or in developing countries. Moreover, and more disturbingly, there have been no innovative advances in contraceptive technology in the past decade, but merely a modest tinkering with the efficacy or applicability of existing methods. There is presently even a disturbing degree of political inertia and industrial disinterest in this area.

Fortunately, there are still those who recognize the problems and have the ability and drive to seek out means of attacking them. One such person is Warren Jones, obstetrician, scientist and committee man, who has lost few opportunities to promote the idea of regulating human fertility by immunological manipulation. Although recent years have seen the publication of a number of reviews concerned with the

IMAGE
UNAVAILABLE FOR
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REASONS

Landowners at home in the eighteenth-century landscape — detail from Gainsborough's painting of Mr and Mrs Robert Andrews, c.1750. (Courtesy of the National Gallery, London.)

topic, this monograph by Jones is the best assessment to date of the variety of approaches being pursued towards such a goal. It is an eminently readable, well balanced account, extensively referenced for the specialist, yet with sufficient background information on the basic reproductive processes and immunological mechanisms to render it comprehensible to a diverse audience of advanced undergraduates and clinicians.

Those familiar with the series of position papers and reports of the WHO Task Force on Immunological Methods of Fertility Control will recognize at once the themes and variations presented in the book. Jones has clearly drawn heavily upon his long association with WHO and its scientific advisors to develop his personal stance. Although consideration is given to all the presently conceivable (and a few barely conceivable) reproductively unique components that might be used in the development of a contraceptive vaccine, emphasis is rightly given to those currently showing most promise — the β subunit of the placental protein hormone chorionic gonadotrophin (hCG), diverse membrane and cytoplasmic elements of the spermatozoon, and the zona pellucida membrane encapsulating the oocyte and early embryo. Herein lies the declared primary aims and also the strength of Jones's book. Rather weaker, largely owing to an attempt to summarize unconfirmed or controversial data, are some minor sections devoted to immunological aspects of the normal reproductive process. Here there are a number of oversimplifications or unwarranted assertions, and the reader should turn to the more extensive and critical reviews to be found elsewhere in the literature.

Fertility regulation covers both inhibition and promotion of reproduction. Although the author is concerned largely with the former, about which far more is known, he gives brief consideration to the problems of overcoming those forms of infertility suspected of having an immunological basis. The recent approach to immunotherapy of recurrent spontaneous abortion is the only omission.

Although not everyone would share the author's optimism that a contraceptive vaccine is not too far round the corner, it would be excessively pessimistic to believe that the continuing endeavours in this field will not ultimately lead to a successful and acceptable procedure for fertility control. Warren Jones's monograph provides a welcome compass that will surely find a use in the hands of those wishing to determine the position and future direction of this most crucial practical application of reproductive immunology. [1]

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Pioneering spirit in Brazil

D.O. Hall

Energy from Alcohol: The Brazilian Experience.

By Harry Rothman, Rod Greenshields and Francisco Rosillo Callé.

University Press of Kentucky: 1983.

Pp. 187. \$20. Published in Britain

under the title **The Alcohol Economy:**

Fuel Ethanol and the Brazilian

Experience. Frances Pinter, £13.75.

LEAD could be removed from petrol tomorrow if the petrol was blended with alcohol at a 10% or greater concentration — with no harm to conventional engines — because of the inherent octane-boosting properties of alcohol. The Brazilians now blend all their petrol with alcohol produced from sugarcane at concentrations of up to 20%, while the Zimbabweans use 15% and the Americans use a 10% mix to produce high-octanes.

In Brazil alcohol has been added to petrol since about 1930. The proportion used has varied depending on the state of the world sugar market and Government directives, and it is only since a concerted national programme was started in 1975 that a 20% alcohol blend has been the upper limit for all the petrol sold. In addition there are now about 750,000 cars running on pure (95%) alcohol, manufactured by the five major international car makers who produce over a million cars per year, and are currently selling 40,000 of the popular pure-alcohol cars per month.

The Brazilian experience with alcohol is very readably portrayed in this book which gives an excellent thumbnail sketch of what has happened in Brazil to make it the world leader in the use, and in expenditure on, biomass energy and applied biotechnology. Government expenditure is over one billion (US) dollars per year with the aim of producing about five billion litres of alcohol in 1983 and ten billion litres by 1987. About 360 new distilleries are either built or in the planning and construction phase. All of this effort is to try to reduce the annual \$10 billion cost of imported oil to run a transport system in a vast country which has few rail links. This oil cost still accounts for over half of Brazil's total export income.

Such a crash programme has inevitably given rise to problems — as one Brazilian bureaucrat has remarked, "Any country that spends \$5 billion on one programme in 8 years is bound to make mistakes". Nevertheless the authors of this book are very frank in their appraisal of the programme and what efforts are being made to counteract the most obvious problems. It helps that one of the authors is a Brazilian research associate and considerable efforts have been made to use Brazilian source

material. However, this is a very rapidly changing field so that with the inevitable delays in book production, the policies and publications of the last 18 months can only be given a three-page summary at the end of the text.

The various problems — land and wealth distribution, pollution, employment, food production, industrial and agricultural policies, local and international financing, costs of production, energy ratios, efficiencies of fermentation, ethanol for the chemical industry and for export — are all covered, though to varying degrees of satisfaction depending on one's biases. The technology of conversion of sugarcane into alcohol is very thoroughly considered, as befits the expertise of one of the authors. However, although it is acknowledged that the production of the feedstock — the sugarcane itself — represents 50% or more of the total cost of the ethanol produced, it is this production phase which is virtually ignored in the book (presumably because it is assumed that so much is already known about the growing of sugarcane and about sugar production). It is recognized that Brazilian yields on average are low (and the conversion efficiencies to alcohol also low) so that a doubling of yields of alcohol per hectare per year should be achievable, thus altering final costs and energy ratios considerably.

Publicity for *Energy from Alcohol* suggests that it is the "first full account of Brazil's development of the alcohol industry, drawing upon many sources unavailable in English". While fully recommending the book, I wouldn't totally agree with this claim. There are a number of important references in English by Brazilian authors and agencies which are not mentioned. Also many readers will find at least a third of the references to be untraceable, and these are nearly all to English-language publications. One can also nigger about some confusing use of terms — gallons (!mperial or US?), dollars in 1980 value or not, gasoline = petrol and not petroleum?, and so on. However, this is compensated for by the useful appendices containing data about Brazil, the properties of alcohol and its blends, and addresses of commercial and government agencies. There is also a useful author and subject index.

The Brazilian experience is unique and is certainly "instructive not only to the industrial nations but to those of the Third World", though this is also true of Zimbabwe and the United States. Nonetheless the Brazilians have by far the highest expenditure for importing and exporting both hardware and technological expertise and have approached the energy problems mostly on their own, much to the scepticism of the rest of the world. They are to be congratulated for such pioneering spirit. [1]

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